Analysis of Variance for FMRI Data

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Abstract

This document describes the programs contained within the *AFNI* distribution for performing Analysis of Variance on collections of *AFNI* 3-dimensional data sets. Specifically, programs 3dANOVA, 3dANOVA2, and 3dANOVA3 (for one, two, and three factor Analysis of Variance, respectively) are described herein.

1 Program 3dANOVA

1.1 Purpose

Program 3dANOVA was developed to perform single-factor analysis of variance on collections of *AFNI* 3-dimensional data sets, voxel by voxel. Through the command line inputs, the user specifies which *AFNI* data sets are to be used in the analysis, and to which factor level they belong. Various output options are available, including the F-test for equality of factor level means, estimation of individual factor level means, estimation of the difference between two factor level means, and estimation of contrasts. The resulting output may be stored either as multiple *AFNI* 2 sub-brick datasets, or as a single *AFNI* "bucket" type dataset.

1.2 Theory

Analysis of variance (ANOVA) is a statistical technique for studying the relation between a dependent variable and one or more independent variables. ANOVA does not make any assumption about the functional relationship of the dependent and independent variables, nor is it assumed that the independent variables are quantitative (i.e., the independent variables may be qualitative).

The independent variables in ANOVA are referred to as *factors*. In this section we consider the case where there is one factor. The different values that the factor takes on in an investigation are the factor "levels" or "treatments". Note that factor levels may be either quantitative or qualitative.

Calculation of ANOVA for FMRI data is greatly complicated by the enormous number of ANOVA's that must be calculated — one for each voxel. This may require the simultaneous calculation of millions of ANOVAs (sequential calculation of the ANOVAs would take forever). In order to process this vast amount of data, the results of intermediate calculations are stored (temporarily) in disk files. These disk files are removed automatically by the program when the results are no longer needed.

We begin with a discussion of the theory behind the ANOVA procedure, providing a very brief summary of the exposition found in the text *Applied Linear Statistical Models* by Neter, Wasserman, and Kutner (publisher: IRWIN, Homewood, IL). This is followed by a description of program 3dANOVA which implements the single factor analysis of variance. A (hypothetical) example is presented to illustrate how the program may be used.

1.2.1 ANOVA model

Here we assume that there is only a single independent variable, or factor. Suppose there are r levels (or *treatments*) for the single-factor under study, and suppose that at factor level i there are n_i observations ($i = 1, ..., r$). (Note that it is not necessary for the sample size to be the same at each level).

$$\begin{array}{cccccc} 1 & 2 & \dots & r & \leftarrow & \text{factor levels} \\ \hline Y_{11} & Y_{21} & \dots & Y_{r1} & \leftarrow & \text{data} \\ Y_{12} & Y_{22} & \dots & Y_{r2} & & \\ \vdots & \vdots & \vdots & \vdots & & \\ Y_{1n_1} & Y_{2n_2} & \dots & Y_{rn_r} & & \end{array}$$

(Here, each Y_{ij} represents an observation at the same voxel across different *AFNI* 3D datasets.)

The total number of observations (i.e., the number of input *AFNI* 3D datasets) is n_t, where

$$n_t = \sum_{i=1}^{r} n_i \ .$$

Then the "fixed-effects" ANOVA model is given by:

$$Y_{ij} = \mu_i + \varepsilon_{ij}, \qquad (i = 1, \dots, r; \quad j = 1, \dots, n_i),$$

where

Y_{ij} = jth observation at factor level i,
μ_i = mean response at factor level i,
ε_{ij} = random variables, iid $N(0, \sigma^2)$.

The total of the observations at factor level i is represented by $Y_{i.}$:

$$Y_{i.} = \sum_{j=1}^{n_i} Y_{ij} \ ,$$

and the total of all observations is denoted $Y_{..}$:

$$Y_{..} = \sum_{i=1}^{r} \sum_{j=1}^{n_i} Y_{ij} \ .$$

The sample mean for factor level i is denoted $\bar{Y}_{i.}$:

$$\bar{Y}_{i.} = \frac{\sum_{j=1}^{n_i} Y_{ij}}{n_i} = \frac{Y_{i.}}{n_i} \ ,$$

and the overall mean is denoted $\bar{Y}_{..}$:

$$\bar{Y}_{..} = \frac{\sum_{i=1}^{r} \sum_{j=1}^{n_i} Y_{ij}}{n_t} = \frac{Y_{..}}{n_t} \ .$$

The deviation of an individual observation from the overall mean is decomposed as follows:

$$\underbrace{Y_{ij} - \bar{Y}_{..}}_{\substack{\text{total deviation} \\ \text{about overall mean}}} = \underbrace{Y_{ij} - \bar{Y}_{i.}}_{\substack{\text{deviation about} \\ \text{factor level mean}}} + \underbrace{\bar{Y}_{i.} - \bar{Y}_{..}}_{\substack{\text{deviation of} \\ \text{factor level mean} \\ \text{about overall mean}}}$$

Squaring both sides, and summing over all observations and all factor levels, we get:

$$\underbrace{\sum_{i=1}^{r} \sum_{j=1}^{n_i} \left(Y_{ij} - \bar{Y}_{..}\right)^2}_{\text{SSTO}} = \underbrace{\sum_{i=1}^{r} \sum_{j=1}^{n_i} \left(Y_{ij} - \bar{Y}_{i.}\right)^2}_{\text{SSE}} + \underbrace{\sum_{i=1}^{r} n_i \left(\bar{Y}_{i.} - \bar{Y}_{..}\right)^2}_{\text{SSTR}} \ ,$$

where we define

$$\begin{array}{lll} \text{SSTO} & = \sum_{i=1}^{r} \sum_{j=1}^{n_i} \left(Y_{ij} - \bar{Y}_{..}\right)^2 & \text{total sum of squares,} \\ \text{SSTR} & = \sum_{i=1}^{r} n_i \left(\bar{Y}_{i.} - \bar{Y}_{..}\right)^2 & \text{treatment sum of squares,} \\ \text{SSE} & = \sum_{i=1}^{r} \sum_{j=1}^{n_i} \left(Y_{ij} - \bar{Y}_{i.}\right)^2 & \text{error sum of squares.} \end{array}$$

The mean squares are obtained by dividing the sum of squares by the corresponding degrees of freedom:

$$\begin{aligned} MSTR &= \frac{SSTR}{r-1} \ , \\ MSE &= \frac{SSE}{n_t - r} \ . \end{aligned}$$

The above can be summarized in an ANOVA table:

Source of Variation	SS	df	MS
Between treatments	$SSTR$	$r-1$	$MSTR = \frac{SSTR}{r-1}$
Within treatments	SSE	$n_t - r$	$MSE = \frac{SSE}{n_t - r}$
Total	$SSTO$	$n_t - 1$	

1.2.2 Test for equality of factor level means

To test the null hypothesis (all factor level means are equal):

$$H_o : \mu_1 = \mu_2 = \cdots = \mu_r$$

against the alternative hypothesis:

$$H_a : \text{ not all } \mu_i \text{ are equal}$$

use the test statistic

$$F^* = \frac{MSTR}{MSE} \ .$$

If F^* is large, this tends to support H_a, whereas if F^* is small, this tends to support H_o. It can be shown that, if H_o is true, then F^* has the $F(r-1, n_t - r)$ distribution.

So, the appropriate decision rule (for significance level α) is:

$$\begin{array}{l} \text{if } F^* \leq F(1-\alpha; r-1; n_t - r), \text{ conclude } H_o \ , \\ \text{if } F^* > F(1-\alpha; r-1; n_t - r), \text{ conclude } H_a \ . \end{array}$$

Once it has been determined that there is a treatment effect, i.e., that not all factor level means are equal, then further investigation is required to determine which treatment(s) are responsible for rejection of the null hypothesis.

1.2.3 Estimation of factor level means

The ith factor level mean μ_i is estimated by

$$\hat{\mu}_i = \bar{Y}_{i.} \ ,$$

and the estimated variance of $\bar{Y}_{i.}$ is denoted $s^2(\bar{Y}_{i.})$,

$$s^2(\bar{Y}_{i.}) = \frac{MSE}{n_i} \ .$$

Then $\dfrac{\bar{Y}_{i.} - \mu_i}{s(\bar{Y}_{i.})}$ is distributed as $t(n_t - r)$. Hence, the $1-\alpha$ confidence limits for μ_i are:

$$\bar{Y}_{i.} \pm t(1 - \frac{\alpha}{2}; n_t - r) s(\bar{Y}_{i.}) \ .$$

Thus, if $t^* = \dfrac{\bar{Y}_{i.}}{s(\bar{Y}_{i.})}$ is large in absolute value, this would tend to indicate that the ith level mean is different from zero.

1.2.4 Estimation of difference between two factor level means

If it has been determined that there is a treatment effect, a natural next step would be to examine pairs of treatments for significant differences. The difference D_{ij} between two factor level means μ_i and μ_j, $D_{ij} = \mu_i - \mu_j$, is estimated by

$$\hat{D}_{ij} = \bar{Y}_{i.} - \bar{Y}_{j.} \ .$$

The estimated variance of $\hat{D}_{ij}$ is given by:

$$s^2(\hat{D}_{ij}) = MSE\left(\frac{1}{n_i} + \frac{1}{n_j}\right) \ .$$

Therefore, $\dfrac{\hat{D}_{ij} - D_{ij}}{s(\hat{D}_{ij})}$ is distributed as $t(n_t - r)$, so the $1-\alpha$ confidence limits for D_{ij} are:

$$\hat{D}_{ij} \pm t(1 - \frac{\alpha}{2}; n_t - r)s(\hat{D}_{ij}) \ .$$

Thus, if $t^* = \dfrac{\hat{D}_{ij}}{s(\hat{D}_{ij})}$ is large in absolute value, this would tend to indicate that the ith and jth factor level means are indeed different.

1.2.5 Estimation of a contrast

As a generalization of the difference between two factor level means, consider: a *contrast* is a linear combination of factor level means, where the coefficients sum to zero:

$$L = \sum_{i=1}^{r} c_i\mu_i, \quad \sum_{i=1}^{r} c_i = 0 \ .$$

The contrast L is estimated by:

$$\hat{L} = \sum_{i=1}^{r} c_i\bar{Y}_{i.} \ ,$$

and the variance of $\hat{L}$ is estimated by:

$$s^2(\hat{L}) = MSE\sum_{i=1}^{r}\frac{c_i^2}{n_i} \ .$$

Thus, $\dfrac{\hat{L} - L}{s(\hat{L})}$ is distributed as $t(n_t - r)$, and the $1-\alpha$ confidence limits for L are:

$$\hat{L} \pm t(1 - \frac{\alpha}{2}; n_t - r)s(\hat{L}) \ .$$

Thus, if $t^* = \dfrac{\hat{L}}{s(\hat{L})}$ is large in absolute value, then we have reason to reject the null hypothesis that the contrast is zero.

1.2.6 Simultaneous inference

The confidence coefficient $1-\alpha$ for the estimated means, pairwise differences, and contrasts mentioned above applies only for a *single estimate*. If multiple comparisons are to be made, the confidence coefficient must be adjusted accordingly. For example, if g contrasts are to be estimated, then by the Bonferroni method the following confidence limits are correct with confidence coefficient $1-\alpha$:

$$\hat{L}_i \pm t(1-\frac{\alpha}{2g}; n_t - r)s(\hat{L}_i), \quad i = 1, \ldots, g \ .$$

However, this applies only for a single voxel. If the *AFNI* 3D data sets each contain N voxels, then the corresponding confidence limits for the entire 3D volume (again using the Bonferroni method) are:

$$\hat{L}_{i,vox} \pm t(1-\frac{\alpha}{2gN}; n_t - r)s(\hat{L}_{i,vox}), \quad i = 1, \ldots, g, \quad vox = 1, \ldots, N \ .$$

1.3 Usage

The command line format for program 3dANOVA is as follows:

3dANOVA -levels *r* \
-dset *1 filename* ... **-dset** *1 filename* \
⋮
-dset *r filename* ... **-dset** *r filename* \
[**-voxel** *num*] \
[**-diskspace**] \
[**-ftr** *prefixname*] \
[**-mean** *i prefixname*] ... [**-mean** *j prefixname*] \
[**-diff** *i j prefixname*] ... [**-diff** *m n prefixname*] \
[**-contr** c_1 c_2 ... c_r *prefixname*] ... [**-contr** d_1 d_2 ... d_r *prefixname*] \
[**-bucket** *prefixname*]

We will explain below what the different command line options do.

1.4 Options

-levels *r*

The -levels command is mandatory, and must appear before any -dset command. The number r following -levels tells the program how many factor levels will be used in the analysis. It is required that r be an integer, $1 < r$ and $r \leq 100$.

-dset *i filename*

The -dset command is used to tell program 3dANOVA which datasets to use in the analysis. The integer i following -dset specifies the factor level for this dataset, and the filename is the name of an *AFNI* 3D dataset. Both the ".HEAD" and ".BRIK" files must be available. Obviously, the integer i must satisfy $1 \le i \le r$, where $r =$ number of factor levels.

There must be a separate -dset command for each data set in the analysis. The data sets may be entered in any order. However, there must be at least 1 data set for each factor level, and the total number of data sets must exceed the number of factor levels. There is a limit of 100 data sets for each factor level. Of course, the data sets must have identical dimensions.

-voxel *num*

The -voxel command instructs program 3dANOVA to display the intermediate ANOVA calculations for the single voxel *num*. This makes it possible to verify the ANOVA calculations for a particular voxel. Since program 3dANOVA takes a while to execute, the -voxel command is useful for reassuring the user that the program has not wandered away.

-diskspace

The -diskspace command tells program 3dANOVA to calculate how much free disk space is required to execute the given task. The amount of disk space required depends upon the dimensions of the input datasets (i.e., number of voxels per image) and the number of output datasets requested. The program prints to the screen how much disk space is required, and a tabulation of the disk space currently available on the system. The program then asks the operator if he wishes to continue.

-ftr *prefixname*

The -ftr command tells 3dANOVA to calculate an F-test for equality of factor level means. Program 3dANOVA calculates the F-statistics for each voxel. The output is then written to the file with the user specified prefix filename. This output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to treatment effect ($\sqrt{MSTR}$) for each voxel, and the second sub-brick contains the corresponding F-statistics. The F-statistics are calculated by dividing the mean sum of squares due to treatment by the mean sum of squares due to error.

$$\textit{AFNI}\ \text{“fift” dataset} \quad \begin{cases} \boxed{I = \sqrt{MSTR}} \\[2ex] \boxed{F^* = \dfrac{MSTR}{MSE}} \end{cases}$$

When this data file is used as input to program *AFNI*, the second sub-brick can be used as a "threshold" for determining which voxels "light-up"; the intensity is then determined by the first sub-brick. The ".HEAD" file for this dataset tells *AFNI* that the second sub-brick contains F-statistics; also, the ".HEAD" file contains the numbers for the numerator degrees of freedom ($df = r - 1$) and the denominator degrees of freedom ($df = n_t - r$). So, by setting the appropriate threshold, only those voxels having the user-specified p-values for significance of the treatment effect will "light-up".

-mean ***i prefixname***
The -mean command is used to estimate the mean of factor level i. Program 3dANOVA calculates the mean and the corresponding t-statistic of the mean for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type “fitt” in the file with the user specified prefix filename. The first sub-brick consists of the estimated mean $\bar{Y}_{i.}$, and the second sub-brick contains the corresponding t-statistic t^* for each voxel.

$$\textit{AFNI}\text{ “fitt” dataset} \quad \begin{cases} \boxed{\bar{Y}_{i.} = \dfrac{\sum_{j=1}^{n_i} Y_{ij}}{n_i}} \\[2ex] \boxed{t^* = \dfrac{\bar{Y}_{i.}}{\sqrt{\dfrac{MSE}{n_i}}}} \end{cases}$$

When this is used as an input file to program *AFNI*, the second sub-brick can be used to set the threshold for determining which voxels have an estimated factor level mean which is significantly different from zero. The “.HEAD” file informs *AFNI* that the second sub-brick contains t-statistics, and that $df = n_t - r$ should be used for the degrees of freedom.

-diff ***i j prefixname***
The -diff command is used to estimate the difference between two factor level means. Program 3dANOVA calculates the difference in the estimated factor means at levels i and j, and the corresponding t-statistic of the difference, for each voxel. The result is stored as a 2 sub-brick “fitt” *AFNI* dataset under the user specified prefix filename. The first sub-brick contains the estimated differences $\hat{D}_{i,j} = \bar{Y}_{i.} - \bar{Y}_{j.}$, and the second sub-brick contains the corresponding t-statistics.

$$\textit{AFNI}\text{ “fitt” dataset} \quad \begin{cases} \boxed{\hat{D}_{i,j} = \bar{Y}_{i.} - \bar{Y}_{j.}} \\[2ex] \boxed{t^* = \dfrac{\hat{D}_{i,j}}{\sqrt{MSE\left(\dfrac{1}{n_i} + \dfrac{1}{n_j}\right)}}} \end{cases}$$

When this file is used as input to program *AFNI*, the second sub-brick can be used to set the threshold for determining which voxels correspond to a significant difference for the ith and jth factor level means. From the “.HEAD” file, *AFNI* knows to use $df = n_t - r$ for the degrees of freedom of the t-statistic.

-contr $c_1\ c_2\ \ldots\ c_r$ ***prefixname***
The -contr command is used to estimate a contrast in factor levels. Program 3dANOVA calculates the estimated contrast $\hat{L} = \sum_{i=1}^{r} c_i \bar{Y}_{i.}$, along with the corresponding t-statistic, for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type “fitt”, under the user specified prefix filename.

$$
AFNI \text{ "fitt" dataset} \begin{cases} \boxed{\hat{L} = \sum_{i=1}^{r} c_i \bar{Y}_{i.}} \\ \\ \boxed{t^* = \dfrac{\hat{L}}{\sqrt{MSE\left(\sum_{i=1}^{r} \dfrac{c_i^2}{n_i}\right)}}} \end{cases}
$$

-bucket *prefixname*

The -bucket command is used in conjunction with the previously described output commands. All of the *AFNI* 2 sub-brick datasets, which are created with the above commands, are replaced with a single *AFNI* "bucket" type dataset. The labels for the individual sub-bricks within the bucket dataset are derived from the user specified prefix filename from the corresponding output command. The individual sub-bricks can be accessed for display within program afni. The purpose of this command is to simplify file management, since all of the output files for a particular problem are now contained within the single *AFNI* bucket dataset. (See Example 2 for an illustration of the use of this command.)

Note that this command requires (the user's selection of) some of the output commands (-ftr, -mean, -diff, -contr) in order to determine which results to calculate, and what labels to apply to the individual sub-bricks.

1.5 Notes

- Since program 3dANOVA uses temporary disk files to hold the results of intermediate calculations, the user should be aware that there must be enough available disk space for the temporary files, in addition to the permanent output files. If the -diskspace command is used, then prior to the start of the ANOVA calculations, program 3dANOVA calculates the maximum amount of disk space required to solve the given problem. Also, the currently available disk space is displayed, and the operator is asked if the program should continue execution.

- The program will not overwrite previously existing files. Therefore, if a file already exists with the same name as an output file specified by the user, the program will terminate. This applies as well to the temporary data files created by the program. All temporary files created by program 3dANOVA have the suffix ".3danova". If, for any reason, the program should prematurely terminate execution, the user must manually remove any ".3danova" files that may be left.

- This program cannot handle complex-valued datasets or time-dependent datasets.

1.6 Examples

Example 1. An investigator is interested in testing whether brain activation patterns differ due to differences in native spoken language. Ten subjects were involved, each having 1 of

4 different native languages. The (hypothetical) *AFNI* 3D data files are stored in directory /user/fred/data/. A sample batch command file for using the 3dANOVA program to analyze the data is presented below:

3dANOVA Batch Command File for Example 1

```
3dANOVA \
-levels 4 \
-dset 1 subj8.lang1+tlrc \
-dset 1 subj1.lang1+tlrc \
-dset 2 subj4.lang2+tlrc \
-dset 2 subj5.lang2+tlrc \
-dset 2 subj0.lang2+tlrc \
-dset 3 subj2.lang3+tlrc \
-dset 3 subj3.lang3+tlrc \
-dset 4 subj7.lang4+tlrc \
-dset 4 subj9.lang4+tlrc \
-dset 4 subj6.lang4+tlrc \
-voxel 1234567 \
-ftr Ftest \
-mean 1 Lang1 \
-mean 2 Lang2 \
-mean 3 Lang3 \
-mean 4 Lang4 \
-diff 2 3 D2-D3 \
-diff 1 2 D1-D2 \
-contr 1.0 1.0 -1.0 -1.0 Cntr1 \
-contr -1.0 -1.0 3.0 -1.0 Cntr2
```

■

In the above example, the user has specified that there are 4 levels, corresponding to the 4 different native languages in the study. There are 10 separate -dset commands which indicate the input files, one for each subject in the study. Of course, both the .HEAD and .BRIK files must be present.

The investigator has requested an F-test for equality of the responses across different languages. The results are to be stored in file name "Ftest+tlrc" (.HEAD and .BRIK) in the current directory. Also, the user has requested an estimate of the mean response for each of the 4 levels (i.e., languages) involved in the study, which results are stored in files "Lang1+tlrc", ..., "Lang4+tlrc" (.HEAD and .BRIK).

Two -diff commands are used; the first conducts a t-test for the difference between language sets 2 and 3; the second -diff command conducts a t-test for the difference between language sets 1 and 2. The results are stored in files D2-D3+tlrc (.HEAD and .BRIK) and D1-D2+tlrc (.HEAD and .BRIK), respectively, in the current directory.

In the above example, the user has requested 2 separate contrast calculations. The first contrast, with $c_1 = 1.0$, $c_2 = 1.0$, $c_3 = -1.0$, and $c_4 = -1.0$, is a test for whether factor levels (i.e., languages) 1 and 2 are significantly different from factor levels 3 and 4. The second contrast, with $c_1 = -1.0$, $c_2 = -1.0$, $c_3 = 3.0$, and $c_4 = -1.0$, is a test for whether language 3 is significantly different from the other languages. The results are stored in files Cntr1+tlrc and Cntr2+tlrc (.HEAD and .BRIK), respectively.

Therefore, a total of $1 + 4 + 2 + 2 = 9$ *AFNI* 3d datasets (18 files) are created by execution of this batch command file.

Example 2. We repeat the above example, but this time the `-bucket` command is appended:

3dANOVA Batch Command File for Example 2

```
3dANOVA \
-levels 4 \
-dset 1 subj8.lang1+tlrc \
-dset 1 subj1.lang1+tlrc \
-dset 2 subj4.lang2+tlrc \
-dset 2 subj5.lang2+tlrc \
-dset 2 subj0.lang2+tlrc \
-dset 3 subj2.lang3+tlrc \
-dset 3 subj3.lang3+tlrc \
-dset 4 subj7.lang4+tlrc \
-dset 4 subj9.lang4+tlrc \
-dset 4 subj6.lang4+tlrc \
-voxel 1234567 \
-ftr Ftest \
-mean 1 Lang1 \
-mean 2 Lang2 \
-mean 3 Lang3 \
-mean 4 Lang4 \
-diff 2 3 L2-L3 \
-diff 1 2 L1-L2 \
-contr 1.0 1.0 -1.0 -1.0 Cntr1 \
-contr -1.0 -1.0 3.0 -1.0 Cntr2 \
-bucket lang.result
```

■

The calculations performed are exactly the same as in Example 1. However, all of the output results are now stored in the single *AFNI* “bucket” dataset “lang.result” (.HEAD and .BRIK), instead of nine separate datasets, as before. The contents of the sub-bricks within the dataset are indicated by their labels:

Brick	Label	Contents	Brick	Label	Contents
#0	Ftest:Inten	$I = \sqrt{MSTR}$	#10	L2-L3:Diff	$\hat{D}_{2,3} = \bar{Y}_{2.} - \bar{Y}_{3.}$
#1	Ftest:F-stat	F-stat for treatment	#11	L2-L3:t-stat	t-stat for $\hat{D}_{2,3}$
#2	Lang1:Mean	$\bar{Y}_{1.} = \frac{1}{n_1}\sum_{j=1}^{n_1} Y_{1j}$	#12	L1-L2:Diff	$\hat{D}_{1,2} = \bar{Y}_{1.} - \bar{Y}_{2.}$
#3	Lang1:t-stat	t-stat for $\bar{Y}_{1.}$	#13	L1-L2:t-stat	t-stat for $\hat{D}_{1,2}$
#4	Lang2:Mean	$\bar{Y}_{2.} = \frac{1}{n_2}\sum_{j=1}^{n_2} Y_{2j}$	#14	Cntr1:Contr	$\hat{L}_1 = \bar{Y}_{1.} + \bar{Y}_{2.}$
#5	Lang2:t-stat	t-stat for $\bar{Y}_{2.}$			$-\bar{Y}_{3.} - \bar{Y}_{4.}$
#6	Lang3:Mean	$\bar{Y}_{3.} = \frac{1}{n_3}\sum_{j=1}^{n_3} Y_{3j}$	#15	Cntr1:t-stat	t-stat for $\hat{L}_1$
#7	Lang3:t-stat	t-stat for $\bar{Y}_{3.}$	#16	Cntr2:Contr	$\hat{L}_2 = -\bar{Y}_{1.} - \bar{Y}_{2.}$
#8	Lang4:Mean	$\bar{Y}_{4.} = \frac{1}{n_4}\sum_{j=1}^{n_4} Y_{4j}$			$+3\bar{Y}_{3.} - \bar{Y}_{4.}$
#9	Lang4:t-stat	t-stat for $\bar{Y}_{4.}$	#17	Cntr2:t-stat	t-stat for $\hat{L}_2$

Note that the sub-bricks are stored in pairs. This follows the usual *AFNI* convention: the first sub-brick in each pair contains some computed quantity or estimate; the second sub-brick in each pair contains the corresponding statistic, which may be used as a display threshold within program afni. Also, note how the labels are derived from the corresponding output commands. These labels are used within afni to identify the individual sub-bricks.

2 Program 3dANOVA2

2.1 Purpose

Program 3dANOVA2 was developed to perform two-factor (or two-way) analysis of variance on *AFNI* 3-dimensional data sets. Through the command line inputs, the user specifies which *AFNI* data sets are to be used in the analysis, and to which factor levels they belong. Various output options are available, including the F-test for factor interaction, F-test for equality of factor level means, estimation of individual factor level means, estimation of the difference between two factor level means, and estimation of contrasts. For the "fixed effects model" (described below), additional output includes estimation of individual cell (treatment) means, differences in cell means, contrasts in cell means, and their corresponding statistics. The resulting output may be stored either as multiple *AFNI* two sub-brick datasets, or as a single *AFNI* "bucket" type dataset.

Program 3dANOVA2 requires **equal sample sizes** for all combinations of factor levels.

2.2 Theory

Analysis of variance (ANOVA) is a statistical technique for studying the relation between a dependent variable and one or more independent variables. ANOVA does not make any assumption about the functional relationship of the dependent and independent variables, nor is it assumed that the independent variables are quantitative (i.e., the independent variables may be qualitative).

The independent variables in ANOVA are referred to as *factors*. In this section we consider the case where there are two factors, each of which may take on different values. The different values that a factor takes on in an investigation are the factor "levels". Each combination of factor levels is a "treatment". Note that factor levels may be either quantitative or qualitative.

Calculation of ANOVA for FMRI data is greatly complicated by the enormous number of ANOVA's that must be calculated — one for each voxel. This may require the simultaneous calculation of millions of ANOVAs (sequential calculation of the ANOVAs would take forever). In order to process this vast amount of data, the results of intermediate calculations are stored (temporarily) in disk files. These disk files are removed automatically by the program when the results are no longer needed.

We begin with a discussion of the theory behind the two-factor ANOVA procedure, providing a very brief summary of the exposition found in the text *Applied Linear Statistical Models* by Neter, Wasserman, and Kutner (publisher: IRWIN, Homewood, IL). This is followed by a description of program 3dANOVA2 which implements the two factor analysis of variance. Some (hypothetical) examples are presented to illustrate how the program may be used.

2.2.1 Fixed effects ANOVA model

We will consider the case where there are two independent variables under study. These independent variables will be referred to as factor A and factor B. Factor A will be studied at a levels, and factor B will be studied at b levels.

$$
\begin{array}{cccccc}
 & 1 & 2 & \ldots & b & \leftarrow \text{ factor B levels} \\
\hline
 & Y_{111} & Y_{121} & & Y_{1b1} & \\
1 & \vdots & \vdots & \cdots & \vdots & \\
 & Y_{11n} & Y_{12n} & & Y_{1bn} & \\
 & Y_{211} & Y_{221} & & Y_{2b1} & \\
2 & \vdots & \vdots & \cdots & \vdots & \\
 & Y_{21n} & Y_{22n} & & Y_{2bn} & \\
\vdots & \vdots & \vdots & \vdots & \vdots & \\
 & Y_{a11} & Y_{a21} & & Y_{ab1} & \\
a & \vdots & \vdots & \cdots & \vdots & \\
 & Y_{a1n} & Y_{a2n} & & Y_{abn} & \\
\uparrow & & & & & \\
\text{factor A levels} & & & & &
\end{array}
$$

(Here, each Y_{ijk} represents an observation at the same voxel across different *AFNI* 3D datasets.)

Throughout this section, we will assume *equal sample sizes*; i.e., for each level i of factor A and for each level j of factor B, there are n observations; so the total number of observations (i.e., number of input *AFNI* 3D datasets) is:

$$n_t = nab.$$

We will initially consider the *fixed-effects* model; for this model, the different levels of factor A and of factor B are of intrinsic interest. Later, we will consider the *random-effects* model and the *mixed effects* model, where the different levels represent a random sample from a much larger population, and the different levels are themselves not of intrinsic interest.

The two-factor "fixed-effects" ANOVA model is:

$$Y_{ijk} = \mu_{ij} + \varepsilon_{ijk}, \qquad (i = 1, \ldots, a; \quad j = 1, \ldots, b; \quad k = 1, \ldots, n),$$

where

Y_{ijk} = kth observation at level i of factor A and level j of factor B,
μ_{ij} = mean response at level i of factor A and level j of factor B;
ε_{ijk} = random variables, iid $N(0, \sigma^2)$.

For a particular cell, i.e., for a given level i of factor A and a given level j of factor B, the sum of the observations within that cell is denoted $Y_{ij.}$:

$$Y_{ij.} = \sum_{k=1}^{n} Y_{ijk} \ .$$

The total of the observations at level i of factor A is represented by $Y_{i..}$:

$$Y_{i..} = \sum_{j=1}^{b} \sum_{k=1}^{n} Y_{ijk} \ .$$

Then the sample mean for level i of factor A is denoted $\bar{Y}_{i..}$:

$$\bar{Y}_{i..} = \frac{\sum_{j=1}^{b} \sum_{k=1}^{n} Y_{ijk}}{nb} = \frac{Y_{i..}}{nb} \ .$$

Similarly, the sum of observations at level j of factor B is given by $Y_{.j.}$:

$$Y_{.j.} = \sum_{i=1}^{a} \sum_{k=1}^{n} Y_{ijk},$$

and the sample mean at level j of factor B is $\bar{Y}_{.j.}$:

$$\bar{Y}_{.j.} = \frac{\sum_{i=1}^{a} \sum_{k=1}^{n} Y_{ijk}}{na} = \frac{Y_{.j.}}{na} \ .$$

The total of all observations is denoted $Y_{..}$:

$$Y_{...} = \sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n} Y_{ijk} \ ,$$

and the overall mean is denoted $\bar{Y}_{...}$:

$$\bar{Y}_{...} = \frac{\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} Y_{ijk}}{n_t} = \frac{Y_{...}}{n_t} \ .$$

The deviation of an individual observation from the overall mean is decomposed as follows:

$$\underbrace{Y_{ijk} - \bar{Y}_{...}}_{\substack{\text{total deviation}\\ \text{about overall mean}}} = \underbrace{Y_{ijk} - \bar{Y}_{ij.}}_{\substack{\text{deviation about}\\ \text{treatment mean}}} + \underbrace{\bar{Y}_{ij.} - \bar{Y}_{..}}_{\substack{\text{deviation of}\\ \text{treatment mean}\\ \text{about overall mean}}}$$

Squaring both sides, and summing over all observations and all factor levels, we get:

$$SSTO = SSE + SSTR \ ,$$

where

$$\begin{aligned}
SSTO &= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}\left(Y_{ijk} - \bar{Y}_{...}\right)^2 \\
SSTR &= n\sum_{i=1}^{a}\sum_{j=1}^{b}\left(\bar{Y}_{ij.} - \bar{Y}_{...}\right)^2 \\
SSE &= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}\left(Y_{ijk} - \bar{Y}_{ij.}\right)^2
\end{aligned}$$

The term $\bar{Y}_{ij.} - \bar{Y}_{...}$ can be further decomposed as follows:

$$\underbrace{\bar{Y}_{ij.} - \bar{Y}_{...}}_{\substack{\text{deviation of}\\ \text{treatment mean}\\ \text{about overall mean}}} = \underbrace{\bar{Y}_{i..} - \bar{Y}_{...}}_{\substack{A \text{ main}\\ \text{effect}}} + \underbrace{\bar{Y}_{.j.} - \bar{Y}_{...}}_{\substack{B \text{ main}\\ \text{effect}}} + \underbrace{\bar{Y}_{ij.} - \bar{Y}_{i..} - \bar{Y}_{.j.} + \bar{Y}_{...}}_{AB \text{ interaction effect}}$$

Squaring both sides, and summing over all observations and all factor levels, we get:

$$SSTR = SSA + SSB + SSAB,$$

where

$$\begin{aligned}
SSA &= nb\sum_{i=1}^{a}(\bar{Y}_{i..} - \bar{Y}_{...})^2 \\
SSB &= na\sum_{j=1}^{b}(\bar{Y}_{.j.} - \bar{Y}_{...})^2 \\
SSAB &= n\sum_{i=1}^{a}\sum_{j=1}^{b}(\bar{Y}_{ij.} - \bar{Y}_{i..} - \bar{Y}_{.j.} + \bar{Y}_{...})^2 \ .
\end{aligned}$$

The mean squares are obtained by dividing the sum of squares by the corresponding degrees of freedom. The above can be summarized in an ANOVA table:

Source of Variation	SS	df	MS
Factor A	SSA	$a-1$	$MSA = \frac{SSA}{a-1}$
Factor B	SSB	$b-1$	$MSB = \frac{SSB}{b-1}$
Interactions	$SSAB$	$(a-1)(b-1)$	$MSAB = \frac{SSAB}{(a-1)(b-1)}$
Between treatments	$SSTR$	$ab-1$	$MSTR = \frac{SSTR}{ab-1}$
Error	SSE	$ab(n-1)$	$MSE = \frac{SSE}{ab(n-1)}$
Total	$SSTO$	$nab-1$	

2.2.2 F-tests

Test for interaction To test for interaction between factors A and B, i.e., to test the null hypothesis

$$H_o : \mu_{ij} - \mu_{i.} - \mu_{.j} + \mu_{..} = 0 \quad \text{for all } i, j$$

against the alternative hypothesis

$$H_a : \mu_{ij} - \mu_{i.} - \mu_{.j} + \mu_{..} \neq 0 \quad \text{for some } i, j$$

use the test statistic

$$F^* = \frac{MSAB}{MSE} \ .$$

When H_o is true, F^* has the $F((a-1)(b-1), ab(n-1))$ distribution. So, the appropriate decision rule (for significance level α) is:

$$\begin{array}{l} \text{if } F^* \leq F(1-\alpha; (a-1)(b-1); ab(n-1)), \text{ conclude } H_o, \\ \text{if } F^* > F(1-\alpha; (a-1)(b-1); ab(n-1)), \text{ conclude } H_a. \end{array}$$

Test for factor A main effect To test whether factor A main effects are present,

$$\begin{array}{rcl} H_o & : & \mu_{1.} = \mu_{2.} = \cdots = \mu_{a.} \\ \text{vs. } H_a & : & \text{not all } \mu_{i.} \text{ are equal} \end{array}$$

use the test statistic

$$F^* = \frac{MSA}{MSE} \ .$$

When H_o is true, F^* has the $F(a-1, ab(n-1))$ distribution. So, the appropriate decision rule (for significance level α) is:

$$\begin{array}{l} \text{if } F^* \leq F(1-\alpha; a-1; ab(n-1)), \text{ conclude } H_o, \\ \text{if } F^* > F(1-\alpha; a-1; ab(n-1)), \text{ conclude } H_a. \end{array}$$

Test for factor B main effect To test whether factor B main effects are present,

$$\begin{aligned} H_o &: \mu_{.1} = \mu_{.2} = \cdots = \mu_{.b} \\ \text{vs. } H_a &: \text{ not all } \mu_{.j} \text{ are equal} \end{aligned}$$

use the test statistic

$$F^* = \frac{MSB}{MSE} \ .$$

When H_o is true, F^* has the $F(b-1, ab(n-1))$ distribution. So, the appropriate decision rule (for significance level α) is:

$$\begin{aligned} &\text{if } F^* \leq F(1-\alpha; b-1; ab(n-1)), \text{ conclude } H_o, \\ &\text{if } F^* > F(1-\alpha; b-1; ab(n-1)), \text{ conclude } H_a. \end{aligned}$$

2.2.3 Estimation of factor level means

The ith level mean of factor A, $\mu_{i.}$, is estimated by

$$\hat{\mu}_{i.} = \bar{Y}_{i..} \ ,$$

and the estimated variance of $\bar{Y}_{i..}$ is denoted $s^2(\bar{Y}_{i..})$,

$$s^2(\bar{Y}_{i..}) = \frac{MSE}{bn} \ .$$

Then $\dfrac{\bar{Y}_{i..} - \mu_{i.}}{s(\bar{Y}_{i..})}$ is distributed as $t(ab(n-1))$. Hence, the $1-\alpha$ confidence limits for $\mu_{i.}$ are:

$$\bar{Y}_{i..} \pm t(1 - \frac{\alpha}{2}; ab(n-1)) s(\bar{Y}_{i..}) \ .$$

Therefore, if $t^* = \dfrac{\bar{Y}_{i..}}{s(\bar{Y}_{i..})}$ is large in absolute value, this would tend to indicate that the ith level mean for factor A is different from zero.

Similarly, the jth level mean of factor B, $\mu_{.j}$, is estimated by

$$\hat{\mu}_{.j} = \bar{Y}_{.j.} \ ,$$

and the estimated variance of $\bar{Y}_{.j.}$ is denoted $s^2(\bar{Y}_{.j.})$,

$$s^2(\bar{Y}_{.j.}) = \frac{MSE}{an} \ .$$

Then $\dfrac{\bar{Y}_{.j.} - \mu_{.j}}{s(\bar{Y}_{.j.})}$ is distributed as $t(ab(n-1))$. Hence, the $1-\alpha$ confidence limits for $\mu_{.j}$ are:

$$\bar{Y}_{.j.} \pm t(1 - \frac{\alpha}{2}; ab(n-1)) s(\bar{Y}_{.j.}) \ .$$

So, if $t^* = \dfrac{\bar{Y}_{.j.}}{s(\bar{Y}_{.j.})}$ is large in absolute value, this would tend to indicate that the jth level mean for factor B is different from zero.

2.2.4 Estimation of difference in factor level means

The difference D_{ij} between two factor A level means $\mu_{i.}$ and $\mu_{j.}$, $D_{ij} = \mu_{i.} - \mu_{j.}$, is estimated by

$$\hat{D}_{ij} = \bar{Y}_{i..} - \bar{Y}_{j..} \ .$$

The estimated variance of $\hat{D}_{ij}$ is given by:

$$s^2(\hat{D}_{ij}) = MSE \left(\frac{2}{bn} \right) \ .$$

Therefore, $\dfrac{\hat{D}_{ij} - D_{ij}}{s(\hat{D}_{ij})}$ is distributed as $t(ab(n-1))$, so the $1-\alpha$ confidence limits for D_{ij} are:

$$\hat{D}_{ij} \pm t(1 - \frac{\alpha}{2}; ab(n-1)) s(\hat{D}_{ij}) \ .$$

Thus, if $t^* = \dfrac{\hat{D}_{ij}}{s(\hat{D}_{ij})}$ is large in absolute value, this would tend to indicate that factor A level means $\mu_{i.}$ and $\mu_{j.}$ are different.

Similarly, the difference D_{ij} between two factor B level means $\mu_{.i}$ and $\mu_{.j}$, $D_{ij} = \mu_{.i} - \mu_{.j}$, is estimated by

$$\hat{D}_{ij} = \bar{Y}_{.i.} - \bar{Y}_{.j.} \ .$$

The estimated variance of $\hat{D}_{ij}$ is given by:

$$s^2(\hat{D}_{ij}) = MSE \left(\frac{2}{an} \right) \ .$$

Therefore, $\dfrac{\hat{D}_{ij} - D_{ij}}{s(\hat{D}_{ij})}$ is distributed as $t(ab(n-1))$, so the $1-\alpha$ confidence limits for D_{ij} are:

$$\hat{D}_{ij} \pm t(1 - \frac{\alpha}{2}; ab(n-1)) s(\hat{D}_{ij}) \ .$$

So, if $t^* = \dfrac{\hat{D}_{ij}}{s(\hat{D}_{ij})}$ is large in absolute value, this would tend to indicate that factor B level means $\mu_{.i}$ and $\mu_{.j}$ are different.

2.2.5 Estimation of contrast of factor level means

A contrast of factor A level means

$$L = \sum_{i=1}^{a} c_i \mu_{i.}, \quad \sum_{i=1}^{a} c_i = 0 \ ,$$

is estimated by:

$$\hat{L} = \sum_{i=1}^{a} c_i \bar{Y}_{i..} \ ,$$

and the variance of $\hat{L}$ is estimated by:

$$s^2(\hat{L}) = \frac{MSE}{bn} \sum_{i=1}^{a} c_i^2 \ .$$

Thus, $\dfrac{\hat{L} - L}{s(\hat{L})}$ is distributed as $t(ab(n-1))$, and the $1-\alpha$ confidence limits for L are:

$$\hat{L} \pm t(1 - \frac{\alpha}{2}; ab(n-1))s(\hat{L}) \ .$$

Therefore, if $t^* = \dfrac{\hat{L}}{s(\hat{L})}$ is large in absolute value, then we have reason to conclude that the contrast L is non-zero.

Similarly, a contrast of factor B level means

$$L = \sum_{j=1}^{b} c_j \mu_{.j}, \quad \sum_{j=1}^{b} c_j = 0 \ .$$

is estimated by:

$$\hat{L} = \sum_{j=1}^{b} c_j \bar{Y}_{.j.} \ ,$$

and the variance of $\hat{L}$ is estimated by:

$$s^2(\hat{L}) = \frac{MSE}{an} \sum_{j=1}^{b} c_j^2 \ .$$

Thus, $\dfrac{\hat{L} - L}{s(\hat{L})}$ is distributed as $t(ab(n-1))$, and the $1-\alpha$ confidence limits for L are:

$$\hat{L} \pm t(1 - \frac{\alpha}{2}; ab(n-1))s(\hat{L}) \ .$$

Again, if $t^* = \dfrac{\hat{L}}{s(\hat{L})}$ is large in absolute value, then we may conclude that the contrast L is non-zero.

2.2.6 Random effects model and mixed effects model

Until now we have only considered the fixed effects model. Now we consider the cases where both factors A and B are random (random effects model) and where factor A is fixed, and factor B is random (mixed effects model). Recall that a factor is fixed if the levels of the factor are of inherent interest, whereas a factor is random if the levels of that factor constitute a random sample from a much larger population, so that the individual levels are not of inherent interest. We again assume equal sample sizes.

The random effects ANOVA model, where both factor A and B have random levels, is:

$$Y_{ijk} = \mu_{..} + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where

$Y_{ijk} =$ kth response at ith level of factor A and jth level of factor B;
$\mu_{..} =$ constant;
$\alpha_i =$ random variable, iid $N(0, \sigma_\alpha^2)$;
$\beta_j =$ random variable, iid $N(0, \sigma_\beta^2)$;
$(\alpha\beta)_{ij} =$ random variable, iid $N(0, \sigma_{\alpha\beta}^2)$;
$\varepsilon_{ijk} =$ random variable, iid $N(0, \sigma^2)$;
α_i, β_j, $(\alpha\beta)_{ij}$, ε_{ijk} are independent.

The calculations for sums of squares, degrees of freedom, and mean squares for the random effects model are the same as for the fixed effects model. The models differ in the appropriate choice for test statistic:

$$F^* = \frac{MSA}{MSAB} \quad \text{for factor } A \text{ main effect,}$$

$$F^* = \frac{MSB}{MSAB} \quad \text{for factor } B \text{ main effect,}$$

$$F^* = \frac{MSAB}{MSE} \quad \text{for interaction.}$$

The mixed effects ANOVA model, where factor A has fixed levels and factor B has random levels, is:

$$Y_{ijk} = \mu_{..} + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where

$Y_{ijk} =$ kth response at ith level of factor A and jth level of factor B;
$\mu_{..} =$ constant;
$\alpha_i =$ constant, subject to $\sum_i \alpha_i = 0$;
$\beta_j =$ random variable, iid $N(0, \sigma_\beta^2)$;
$(\alpha\beta)_{ij} =$ random variable, $N(0, \frac{a-1}{a}\sigma_{\alpha\beta}^2)$, subject to $\sum_i (\alpha\beta)_{ij} = 0$ for all j;
$\varepsilon_{ijk} =$ random variable, iid $N(0, \sigma^2)$;
β_j, $(\alpha\beta)_{ij}$, ε_{ijk} are independent.

The calculations for sums of squares, degrees of freedom, and mean squares for the mixed effects model are the same as for the fixed effects model. The models differ in the appropriate choice for test statistic:

$$F^* = \frac{MSA}{MSAB} \quad \text{for factor } A \text{ main effect,}$$

$$F^* = \frac{MSB}{MSE} \quad \text{for factor } B \text{ main effect,}$$

$$F^* = \frac{MSAB}{MSE} \quad \text{for interaction.}$$

In each case, the numerator and denominator degrees of freedom are the same as those for the corresponding mean square.

2.2.7 Simultaneous inference

The discussion for program 3dANOVA concerning the appropriate confidence levels when multiple comparisons are made applies here as well.

2.3 Usage

The command line format for program 3dANOVA2 is as follows:

3dANOVA2 -type *k* **-alevels** *a* **-blevels** *b* \
-dset *1 1 filename* ... **-dset** *1 1 filename* \
⋮
-dset *a b filename* ... **-dset** *a b filename* \
[**-voxel** *num*] \
[**-diskspace**] \
[**-ftr** *prefixname*] \
[**-fa** *prefixname*] [**-fb** *prefixname*] [**-fab** *prefixname*] \
[**-amean** *i prefixname*] [**-bmean** *j prefixname*] [**-xmean** *i j prefixname*] \
[**-adiff** *i j prefixname*] [**-bdiff** *i j prefixname*] [**-xdiff** *i j k l prefixname*] \
[**-acontr** $c_1 \ldots c_a$ *prefixname*] [**-bcontr** $c_1 \ldots c_b$ *prefixname*] \
[**-xcontr** $c_{11} \ldots c_{1b}\ c_{21} \ldots c_{2b} \ldots c_{a1} \ldots c_{ab}$] \
[**-bucket** *prefixname*]

2.4 Options: Fixed effects model

-type *k*

The -type command is used to indicate which specific ANOVA model should be used, depending on the integer k following -type. If $k = 1$, then the "fixed effects" model is used. This is the default option, i.e., unless the -type command appears, the program will automatically assume that the "fixed effects" model is appropriate. If $k = 2$, program 3dANOVA2 uses the "random effects" model. For the "random effects" model, the levels of factor A and the levels of factor B are treated as random samples from a much larger population. The last option, $k = 3$, indicates that the "mixed effects" model is to be used. For the "mixed effects" model, program 3dANOVA2 assumes that factor A is "fixed", and that factor B is "random", i.e., the levels of factor B constitute a random sample.

In this section, we will assume that a fixed effects model is being used.

-alevels *a*

The -alevels command tells program 3dANOVA2 that a levels will be used for factor A. The number a following -alevels must be an integer, $1 < a \leq 100$. The -alevels command is mandatory, and must appear before any -dset command.

-blevels *b*

The -blevels command indicates that b levels will be used for factor B. The number b following -blevels must be an integer, $1 < b \leq 100$. The -blevels command is mandatory, and must appear before any -dset command.

-dset *i j filename*

The -dset command is used to tell program 3dANOVA2 which datasets to use in the analysis. The first number, i, which follows -dset specifies the level of factor A for this dataset, and the second number, j, specifies the level of factor B. The last item, "filename", is the name of an *AFNI* 3D dataset. Both the ".HEAD" and ".BRIK" files must be available. Obviously, the integers i and j must satisfy $1 \leq i \leq a$ and $1 \leq j \leq b$, where $a =$ number of levels of factor A, and $b =$ number of levels of factor B.

There must be a separate -dset command for each data set in the analysis. The data sets may be entered in any order. It is required that there be an equal number of data sets for each combination of levels for factors A and B. Of course, the data sets must have identical dimensions.

-voxel *num*

The -voxel command instructs program 3dANOVA2 to display the intermediate ANOVA calculations for the single voxel *num*. This makes it possible to verify the ANOVA calculations for a particular voxel. Since program 3dANOVA2 takes a while to execute, the -voxel command is useful for reassuring the user that the program has not wandered away.

-diskspace

The -diskspace command tells program 3dANOVA2 to calculate how much free disk space is required to execute the given task. The amount of disk space required depends upon the dimensions of the input datasets (i.e., number of voxels per image) and the number of output datasets requested. The program prints to the screen how much disk space is required, and a tabulation of the disk space currently available on the system. The program then asks the operator if he wishes to continue.

-ftr *prefixname*

The -ftr command tells 3dANOVA2 to calculate an F-test for treatment effect. The output is then written to the file with the user specified prefix filename. The output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to interaction ($\sqrt{MSTR}$) for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$\textit{AFNI}\text{ “fift” dataset} \quad \left\{ \begin{array}{l} \boxed{I = \sqrt{MSTR}} \\ \\ \boxed{F^* = \dfrac{MSTR}{MSE}} \end{array} \right.$$

The ".HEAD" file for this dataset tells *AFNI* that the second sub-brick contains F-statistics with numerator degrees of freedom $df = ab - 1$ and denominator degrees of freedom $df = ab(n-1)$.

-fa *prefixname*
The -fa command tells 3dANOVA2 to calculate an F-test for equality of level means for factor A. The output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to factor A effect ($\sqrt{MSA}$) for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$\textit{AFNI}\ \text{"fift" dataset} \quad \begin{cases} \boxed{I = \sqrt{MSA}} \\ \\ \boxed{F^* = \dfrac{MSA}{MSE}} \end{cases}$$

The ".HEAD" file for this dataset tells *AFNI* that the second sub-brick contains F-statistics with numerator degrees of freedom $df = a-1$ and denominator degrees of freedom $df = ab(n-1)$.

-fb *prefixname*
The -fb command tells 3dANOVA2 to calculate an F-test for equality of level means for factor B. The output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to factor B effect ($\sqrt{MSB}$) for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$\textit{AFNI}\ \text{"fift" dataset} \quad \begin{cases} \boxed{I = \sqrt{MSB}} \\ \\ \boxed{F^* = \dfrac{MSB}{MSE}} \end{cases}$$

The ".HEAD" file for this dataset tells AFNI that the second sub-brick contains F-statistics with numerator degrees of freedom $df = b-1$ and denominator degrees of freedom $df = ab(n-1)$.

-fab *prefixname*
The -fab command tells 3dANOVA2 to calculate an F-test for interaction effect of factors A and B. The output consists of a 2 sub-brick AFNI dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to interaction ($\sqrt{MSAB}$) for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$\textit{AFNI}\ \text{"fift" dataset} \quad \begin{cases} \boxed{I = \sqrt{MSAB}} \\ \\ \boxed{F^* = \dfrac{MSAB}{MSE}} \end{cases}$$

The ".HEAD" file for this dataset tells *AFNI* that the second sub-brick contains F-statistics with numerator degrees of freedom $df = (a-1)(b-1)$ and denominator degrees of freedom $df = ab(n-1)$.

-amean *i prefixname*

The -amean command is used to estimate the mean of level i for factor A. Program 3dANOVA2 calculates the mean and the corresponding t-statistic of the mean for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt" in a file with the user specified prefix filename. The first sub-brick consists of the estimated mean $\bar{Y}_{i..}$, and the second sub-brick contains the corresponding t-statistic t^* for each voxel.

$$\text{\textit{AFNI} ``fitt'' dataset} \quad \left\{ \begin{array}{l} \boxed{\bar{Y}_{i..} = \dfrac{\sum_{j=1}^{b}\sum_{k=1}^{n} Y_{ijk}}{nb}} \\ \\ \boxed{t^* = \dfrac{\bar{Y}_{i..}}{\sqrt{\dfrac{MSE}{nb}}}} \end{array} \right.$$

-bmean *j prefixname*

The -bmean command is used to estimate the mean of level j for factor B. Program 3dANOVA2 calculates the mean and the corresponding t-statistic of the mean for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt" in a file with the user specified prefix filename. The first sub-brick consists of the estimated mean $\bar{Y}_{.j.}$, and the second sub-brick contains the corresponding t-statistic t^* for each voxel.

$$\text{\textit{AFNI} ``fitt'' dataset} \quad \left\{ \begin{array}{l} \boxed{\bar{Y}_{.j.} = \dfrac{\sum_{i=1}^{a}\sum_{k=1}^{n} Y_{ijk}}{na}} \\ \\ \boxed{t^* = \dfrac{\bar{Y}_{.j.}}{\sqrt{\dfrac{MSE}{na}}}} \end{array} \right.$$

-xmean *i j prefixname*

The -xmean command is used to estimate the mean of the cell (or treatment) at level i of factor A and level j of factor B. Program 3dANOVA2 calculates the mean and the corresponding t-statistic of the mean for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt" in a file with the user specified prefix filename. The first sub-brick consists of the estimated mean $\bar{Y}_{ij.}$, and the second sub-brick contains the corresponding t-statistic t^* for each voxel.

$$
\textit{AFNI}\ \text{“fitt” dataset}\quad \begin{cases} \boxed{\bar{Y}_{ij.} = \dfrac{\sum_{k=1}^{n} Y_{ijk}}{n}} \\ \\ \boxed{t^* = \dfrac{\bar{Y}_{ij.}}{\sqrt{\dfrac{MSE}{n}}}} \end{cases}
$$

-adiff *i j prefixname*

The -adiff command is used to estimate the difference between two level means of factor A. Program 3dANOVA2 calculates the difference in the estimated factor A means at levels i and j, and the corresponding t-statistic of the difference, for each voxel. The result is stored as a 2 sub-brick "fitt" *AFNI* dataset under the user specified prefix filename. The first sub-brick contains the estimated differences $\hat{D}_{i,j} = \bar{Y}_{i..} - \bar{Y}_{j..}$, and the second sub-brick contains the corresponding t-statistics.

$$
\textit{AFNI}\ \text{"fitt" dataset}\quad \begin{cases} \boxed{\hat{D}_{i,j} = \bar{Y}_{i..} - \bar{Y}_{j..}} \\ \\ \boxed{t^* = \dfrac{\hat{D}_{i,j}}{\sqrt{MSE\left(\dfrac{2}{bn}\right)}}} \end{cases}
$$

-bdiff *i j prefixname*

The -bdiff command is used to estimate the difference between two level means of factor B. Program 3dANOVA2 calculates the difference in the estimated factor B means at levels i and j, and the corresponding t-statistic of the difference, for each voxel. The result is stored as a 2 sub-brick "fitt" *AFNI* dataset under the user specified prefix filename. The first sub-brick contains the estimated differences $\hat{D}_{i,j} = \bar{Y}_{.i.} - \bar{Y}_{.j.}$, and the second sub-brick contains the corresponding t-statistics.

$$
\textit{AFNI}\ \text{“fitt” dataset}\quad \begin{cases} \boxed{\hat{D}_{i,j} = \bar{Y}_{.i.} - \bar{Y}_{.j.}} \\ \\ \boxed{t^* = \dfrac{\hat{D}_{i,j}}{\sqrt{MSE\left(\dfrac{2}{an}\right)}}} \end{cases}
$$

-xdiff *i j k l prefixname*

The -xdiff command is used to estimate the difference between the mean of the cell at level i of factor A, level j of factor B, and the mean of the cell at level k of factor A, level l of factor B. Program 3dANOVA2 calculates the difference in the cell means, and the corresponding t-statistic of the difference, for each voxel. The result is stored as a 2 sub-brick "fitt" *AFNI* dataset under the user specified prefix filename. The first sub-brick contains the estimated differences $\hat{D}_{i,j,k,l} = \bar{Y}_{ij.} - \bar{Y}_{kl.}$, and the second sub-brick contains the corresponding t-statistics.

$$\textit{AFNI}\ \text{“fitt” dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{D}_{i,j,k,l} = \bar{Y}_{ij.} - \bar{Y}_{kl.}} \\ \\ \boxed{t^* = \dfrac{\hat{D}_{i,j,k,l}}{\sqrt{MSE\left(\dfrac{2}{n}\right)}}} \end{array} \right.$$

-acontr $c_1 ... c_a$ ***prefixname***

The -acontr command is used to estimate a contrast in levels of factor A. Program 3dANOVA2 calculates the estimated contrast $\hat{L} = \sum_{i=1}^{a} c_i \bar{Y}_{i..}$, along with the corresponding t-statistic, for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt", under the user specified prefix filename.

$$\textit{AFNI}\ \text{“fitt” dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{L} = \sum_{i=1}^{a} c_i \bar{Y}_{i..}} \\ \\ \boxed{t^* = \dfrac{\hat{L}}{\sqrt{\dfrac{MSE}{bn}\left(\sum_{i=1}^{a} c_i^2\right)}}} \end{array} \right.$$

-bcontr $c_1 ... c_b$ ***prefixname***

The -bcontr command is used to estimate a contrast in levels of factor B. Program 3dANOVA2 calculates the estimated contrast $\hat{L} = \sum_{j=1}^{b} c_j \bar{Y}_{.j.}$, along with the corresponding t-statistic, for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt", under the user specified prefix filename.

$$\textit{AFNI}\ \text{“fitt” dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{L} = \sum_{j=1}^{b} c_j \bar{Y}_{.j.}} \\ \\ \boxed{t^* = \dfrac{\hat{L}}{\sqrt{\dfrac{MSE}{an}\left(\sum_{j=1}^{b} c_j^2\right)}}} \end{array} \right.$$

-xcontr $c_{11} \ldots\ c_{1b}\ c_{21} \ldots c_{2b}\ \ldots\ c_{a1} \ldots c_{ab}$ ***prefixname***

The -xcontr command is used to estimate a contrast in cell means. Program 3dANOVA2 calculates the estimated contrast $\hat{L} = \sum_{i=1}^{a} \sum_{j=1}^{b} c_{ij} \bar{Y}_{ij.}$, along with the corresponding t-statistic, for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt", under the user specified prefix filename.

$$
AFNI \text{ ``fitt'' dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{L} = \sum_{i=1}^{a} \sum_{j=1}^{b} c_{ij} \bar{Y}_{ij.}} \\ \\ \boxed{t^* = \dfrac{\hat{L}}{\sqrt{\dfrac{MSE}{n} \left(\sum_{i=1}^{a} \sum_{j=1}^{b} c_{ij}^2 \right)}}} \end{array} \right.
$$

-bucket *prefixname*

The -bucket command is used in conjunction with the previously described output commands. All of the *AFNI* 2 sub-brick datasets, which are created with the above commands, are replaced with a single *AFNI* "bucket" type dataset. The labels for the individual sub-bricks within the bucket dataset are derived from the user specified prefix filenames from the corresponding output commands. The individual sub-bricks can be accessed for display within program afni. The purpose of this command is to simplify file management, since all of the output files for a particular problem are now contained within the single *AFNI* bucket dataset. (See Example 1 for an illustration of the use of this command.)

Note that this command requires (the user's selection of) some of the output commands (-ftr, -fa, -fb, -fab, -amean, -bmean, -xmean, -adiff, -bdiff, -xdiff, -acontr, -bcontr, -xcontr) in order to determine which results to calculate, and what labels to apply to the individual sub-bricks.

2.5 Options: Mixed effects model

In this section, we will assume that a mixed effects model is appropriate.

-type *k*

The mixed effects model is selected by using the command -type 3. As mention above, this model is appropriate when the levels of factor A are "fixed", and the levels of factor B are "random" (i.e., the levels of factor B constitute a random sample from a larger population).

-fa *prefixname*

For the mixed effects model, the 2 sub-brick *AFNI* dataset of type "fift" which is generated by the -fa command is as follows:

$$
AFNI \text{ ``fift'' dataset} \quad \left\{ \begin{array}{l} \boxed{I = \sqrt{MSA}} \\ \\ \boxed{F^* = \dfrac{MSA}{MSAB}} \end{array} \right.
$$

Note that when the mixed effects model is specified, the denominator of the F^* statistic, for testing for the presence of factor A main effect, is $MSAB$, and *not* MSE, as was the case with the fixed effects model. Also, the ".HEAD" file for this dataset tells *AFNI* that the second sub-brick contains F-statistics with numerator degrees of freedom $df = a - 1$ and denominator degrees of freedom $df = (a-1)(b-1)$ (the degrees of freedom associated with $MSAB$).

Other options Other options involving factor A (such as -amean, -adiff, and -acontr) also use $MSAB$ for the estimate of mean square error under the mixed effects model. For these options, one observation per cell is the minimum sample size.

The command -fb yields the test statistic $F^* = MSB/MSE$, and -fab yields the test statistic $F^* = MSAB/MSE$. For these options, two observations per cell is the minimum sample size.

Output options -bmean, -xmean, -bdiff, -xdiff, -bcontr, -xcontr and -ftr are not available for the mixed effects model, as they do not seem necessary or appropriate when factor B is random.

2.6 Options: Random effects model

The random effects model is selected by using the command -type 2.

The command -fa yields the test statistic $F^* = MSA/MSAB$, the command -fb yields the test statistic $F^* = MSB/MSAB$. For these options, there must be a minimum of one observation per cell.

The command -fab produces the test statistic $F^* = MSAB/MSE$. For this option, there must be at least two observations per cell.

Output options -amean, -bmean, -xmean, -adiff, -bdiff, -xdiff, -acontr, -bcontr, -xcontr, and -ftr are not available for the random effects model, as they do not seem necessary or appropriate when both factors A and B are random.

2.7 Notes

- Since program 3dANOVA2 uses temporary disk files to hold the results of intermediate calculations, the user should be aware that there must be enough available disk space for the temporary files, in addition to the permanent output files. If the -diskspace command is used, then prior to the start of the ANOVA calculations, program 3dANOVA2 calculates the maximum amount of disk space required to solve the given problem. Also, the currently available disk space is displayed, and the operator is asked if the program should continue execution.

- The program will not overwrite previously existing files. Therefore, if a file already exists with the same name as an output file specified by the user, the program will terminate. This applies as well to the temporary data files created by the program. All temporary files created by program 3dANOVA2 have the suffix ".3danova2". If, for any reason, the program should prematurely terminate execution, the user must manually remove any ".3danova2" files that may be left.

- This program cannot handle complex-valued datasets or time-dependent datasets.

2.8 Examples

Example 1. (Fixed effects model) A researcher wishes to study differences in neural activation arising from two factors: intelligence, and right- vs. left- handedness. Factor A (intelligence) is evaluated at 3 levels: low IQ, medium IQ, and high IQ. Factor B has 2 levels: left-handed, and right-handed. For each combination of IQ level and handedness, 4 subjects are studied, for a total of 24 observations. To evaluate the results, program 3dANOVA2 was used, with the following batch command file.

Batch command file for Example 1

```
3dANOVA2 \
-type 1 \
-alevels 3 \
-blevels 2 \
-dset 1 1 subj13284+tlrc \
-dset 1 1 subj21224+tlrc \
-dset 1 1 subj99052+tlrc \
-dset 1 1 subj00199+tlrc \
-dset 2 1 subj60578+tlrc \
-dset 2 1 subj91240+tlrc \
-dset 2 1 subj97458+tlrc \
-dset 2 1 subj35249+tlrc \
. . .
-dset 3 2 subj38980+tlrc \
-dset 3 2 subj10750+tlrc \
-dset 3 2 subj36247+tlrc \
-dset 3 2 subj70994+tlrc \
-voxel 1234567 \
-ftr Trtmnt \
-fa IQeffct \
-fb Heffct \
-fab IQxH \
-amean 1 IQlow \
-amean 2 IQmed \
-amean 3 IQhigh \
-bmean 1 Hleft \
-bmean 2 Hright \
-adiff 1 2 IQ1-IQ2 \
-adiff 2 3 IQ2-IQ3 \
-adiff 1 3 IQ1-IQ3 \
-acontr -0.5 -0.5 1.0 IQcontr1 \
```

-acontr 1.0 –0.5 -0.5 IQcontr2

■

In this example, the investigator has used the command -type 1, indicating that a "fixed effects" model is appropriate. For both factor A and factor B, the factor levels are of intrinsic interest.

In the above example, there are 3 levels for the factor "intelligence", and so the command -alevels 3 is used. Also, that there are two values for "handedness" is indicated by the command -blevels 2.

In this example, there are 4 data sets for each of the 3 levels of factor A and 2 levels of factor B, requiring 24 separate -dset commands.

In the above batch command file, the investigator has requested an F-test for treatment effect with the -ftr command. The results are to be stored in file name "Trtmnt+tlrc" (.HEAD and .BRIK) in the current directory.

The investigator has requested an F-test for the factor A (intelligence) effect by using the -fa command. The results are to be stored in file name "IQeffct+tlrc" (.HEAD and .BRIK) in the current directory.

An F-test for factor B (handedness) effect is generated by the -fb command. The results are to be stored in file name "Heffct+tlrc" (.HEAD and .BRIK) in the current directory. Also, an F-test for the interaction effect between "intelligence" and "handedness" is produced by the -fab command. The results are to be stored in file name "IQxH+tlrc" (.HEAD and .BRIK) in the current directory.

In Example 1, the user has requested an estimate of the mean response for each of the 3 levels of factor A (IQ level) involved in the study, and an estimate of the mean response for each of the 2 levels of factor B (handedness), using the -amean and -bmean commands, respectively.

Separate tests for significant differences in response between low and medium IQ, medium and high IQ, and low and high IQ, are accomplished by means of the 3 -adiff commands, respectively. The results are written to files "IQ1-IQ2+tlrc", "IQ2-IQ3+tlrc", and "IQ1-IQ3+tlrc" (.HEAD and .BRIK) in the current directory.

Finally, the user has requested 2 separate contrast calculations. The first contrast, with $c_1 = -0.5$, $c_2 = -0.5$, and $c_3 = 1.0$, is a test for whether factor level 3 (high IQ) yields a significantly different result from the combined levels 1 and 2 (low and medium IQ). The second contrast, with $c_1 = 1.0$, $c_2 = -0.5$, and $c_3 = -0.5$, is a test for whether the low IQ results are significantly different from the combined medium and high IQ results.

Therefore, a total of 14 separate *AFNI* 3d datasets (28 files) are created by execution of this batch command file. To simplify matters, all of these datasets can be grouped into a single AFNI "bucket" dataset by appending the -bucket command, as shown below.

Batch command file for Example 1

```
3dANOVA2 \
-type 1 \
-alevels 3 \
```

```
-blevels 2 \
        ⋮
(same as above)
        ⋮
-ftr Trtmnt \
-fa IQeffct \
-fb Heffct \
-fab IQxH \
-amean 1 IQlow \
-amean 2 IQmed \
-amean 3 IQhigh \
-bmean 1 Hleft \
-bmean 2 Hright \
-adiff 1 2 IQ1-IQ2 \
-adiff 2 3 IQ2-IQ3 \
-adiff 1 3 IQ1-IQ3 \
-acontr -0.5 -0.5 1.0 IQcntr1 \
-acontr 1.0 –0.5 -0.5 IQcntr2 \
-bucket test.results
```

■

The calculations performed are exactly the same as above. However, all of the output results are now stored in the *AFNI* "bucket" dataset "test.results" (.HEAD and .BRIK). The contents of the sub-bricks within the dataset are indicated by their labels:

Brick	Label	Contents	Brick	Label	Contents
#0	Trtmnt:Inten	$I = \sqrt{MSTR}$	#14	Hleft:mean	$\bar{Y}_{.1.} = \frac{1}{na}\sum_i \sum_k Y_{i1k}$
#1	Trtmnt:F-stat	F-stat for treatment	#15	Hleft:t-stat	t-stat for $\bar{Y}_{.1.}$
#2	IQeffct:Inten	$I = \sqrt{MSA}$	#16	Hright:mean	$\bar{Y}_{.2.} = \frac{1}{na}\sum_i \sum_k Y_{i2k}$
#3	IQeffct:F-stat	F-stat for IQ	#17	Hright:t-stat	t-stat for $\bar{Y}_{.2.}$
#4	Heffct:Inten	$I = \sqrt{MSB}$	#18	IQ1-IQ2:Diff	$\hat{D}_{1,2} = \bar{Y}_{1..} - \bar{Y}_{2..}$
#5	Heffct:F-stat	F-stat for handedness	#19	IQ1-IQ2:t-stat	t-stat for $\hat{D}_{1,2}$
#6	IQxH:Inten	$I = \sqrt{MSAB}$	#20	IQ2-IQ3:Diff	$\hat{D}_{2,3} = \bar{Y}_{2..} - \bar{Y}_{3..}$
#7	IQxH:F-stat	F-stat for interaction	#21	IQ2-IQ3:t-stat	t-stat for $\hat{D}_{2,3}$
#8	IQlow:mean	$\bar{Y}_{1..} = \frac{1}{nb}\sum_j \sum_k Y_{1jk}$	#22	IQ1-IQ3:Diff	$\hat{D}_{1,3} = \bar{Y}_{1..} - \bar{Y}_{3..}$
#9	IQlow:t-stat	t-stat for $\bar{Y}_{1.}$	#23	IQ1-IQ3:t-stat	t-stat for $\hat{D}_{1,3}$
#10	IQmed:mean	$\bar{Y}_{2..} = \frac{1}{nb}\sum_j \sum_k Y_{2jk}$	#24	IQcntr1:Contr	$\hat{L}_1 = \bar{Y}_{3.} - \frac{\bar{Y}_{1.}+\bar{Y}_{2.}}{2}$
#11	IQmed:t-stat	t-stat for $\bar{Y}_{2.}$	#25	IQcntr1:t-stat	t-stat for $\hat{L}_1$
#12	IQhigh:mean	$\bar{Y}_{3..} = \frac{1}{nb}\sum_j \sum_k Y_{3jk}$	#26	IQcntr2:Contr	$\hat{L}_2 = \bar{Y}_{1.} - \frac{\bar{Y}_{2.}+\bar{Y}_{3.}}{2}$
#13	IQhigh:t-stat	t-stat for $\bar{Y}_{3.}$	#27	IQcntr2:t-stat	t-stat for $\hat{L}_2$

Note that the sub-bricks are stored in pairs. This follows the usual *AFNI* convention: the first sub-brick in each pair contains some computed quantity or estimate; the second sub-brick in each pair contains the corresponding statistic, which may be used as a display threshold within program afni. Also, note how the labels are derived from the corresponding output commands. These labels are used within afni to identify the individual sub-bricks.

Example 2. (Mixed effects model) A researcher wishes to compare the effects of 4 different drugs on neural activation. Each of the drugs will be administered to 20 subjects, and the resulting activation maps will be compared to determine whether the drugs produce significantly different activation. For this study, factor A is the drug effect. The levels of factor A are "fixed", i.e., the levels are of inherent interest. The levels of factor B correspond to the different people in the study. The levels are factor B are "random", i.e., the different levels constitute a random sample from a much larger population, and so the different levels do not have intrinsic significance.

Batch command file for Example 2

```
3dANOVA2 \
-type 3 \
-alevels 4 \
-blevels 20 \
-dset 1 1 subj01.drug1+tlrc \
-dset 2 1 subj01.drug2+tlrc \
-dset 3 1 subj01.drug3+tlrc \
-dset 4 1 subj01.drug4+tlrc \
-dset 1 2 subj02.drug1+tlrc \
-dset 2 2 subj02.drug2+tlrc \
-dset 3 2 subj02.drug3+tlrc \
-dset 4 2 subj02.drug4+tlrc \
. . .
-dset 1 20 subj20.drug1+tlrc \
-dset 2 20 subj20.drug2+tlrc \
-dset 3 20 subj20.drug3+tlrc \
-dset 4 20 subj20.drug4+tlrc \
-fa DrugEffct \
-amean 1 Drug1 \
-amean 2 Drug2 \
-amean 3 Drug3 \
-amean 4 Drug4 \
-adiff 1 2 D1-D2 \
-adiff 1 3 D1-D3 \
-adiff 1 4 D1-D4 \
-adiff 2 3 D2-D3 \
-adiff 2 4 D2-D4 \
-adiff 3 4 D3-D4 \
-acontr -1.0 -1.0 1.0 1.0 Dcntr1 \
```

-acontr 3.0 –1.0 -1.0 -1.0 Dcntr2 \
-bucket Drug.results

■

In Example 2, the investigator has used the command `-type 3`, indicating that a "mixed effects" model is appropriate. Note that since this is a "mixed effects" model, it is possible to have only $n = 1$ samples per cell. Factor A, whose levels correspond to the different drugs used in the experiment, is obviously a fixed effect, since the response due to different drugs is of inherent interest. Factor B, the different subjects used in the experiment, is a random effect. The subjects used in the experiment constitute a random sample from the entire population; the individual responses are not of intrinsic interest.

Since the user included the `-bucket` command, all of the results are stored in the single *AFNI* "bucket" type dataset "Drug.results+tlrc" (.HEAD and .BRIK).

3 Program 3dANOVA3

3.1 Purpose

Program 3dANOVA3 was developed to perform crossed and crossed-nested three factor analysis of variance (ANOVA) on *AFNI* 3-dimensional data sets. Five different ANOVA models are available, which are described below. Although these models do not cover every possible situation for three factor ANOVA, they should suffice for most practical purposes.

The command line inputs allow the user to specify which *AFNI* data sets are to be used in the analysis, and to which factor levels they belong. Various output options are available, including F-tests for main factor effects, F-tests for factor interactions, estimation of individual factor level means, estimation of the difference between two factor level means, and estimation of contrasts. For the "fixed effects model" (Model 1, described below), additional output includes estimation of individual cell (treatment) means, and differences in cell means, along with their corresponding statistics. The resulting output may be stored either as multiple *AFNI* 2 sub-brick datasets, or as a single *AFNI* "bucket" type dataset.

Program 3dANOVA3 requires **equal sample sizes** for all combinations of factor levels.

3.2 Theory

We begin with a discussion of the theory behind the crossed and crossed-nested three factor ANOVA procedure, providing a very brief summary of the exposition found in the text *Applied Linear Statistical Models* by Neter, Wasserman, and Kutner (publisher: IRWIN, Homewood, IL). This is followed by a description of program 3dANOVA3 which implements the three factor analysis of variance. Some (hypothetical) examples are presented to illustrate how the program may be used.

3.2.1 Three factor ANOVA models

We consider experiments where there are three independent variables under study. The factors in the study, which are referred to as factors A, B, and C, are to be studied at a, b, and c levels, respectively. We will assume *equal sample sizes*; i.e., for each level i of factor A, for each level j of factor B, and for each level k of factor C, there are n observations; so the total number of observations (i.e., the number of input *AFNI* 3D datasets) is:

$$n_t = abcn.$$

This experimental layout is illustrated below.

$$
\begin{array}{c|ccc|ccc|c|ccc|l}
 & & 1 & & & 2 & & \cdots & & b & & \leftarrow \text{factor B} \\
\hline
 & Y_{1111} & & Y_{111n} & Y_{1211} & & Y_{121n} & & Y_{1b11} & & Y_{1b1n} & \\
1 & \vdots & \cdots & \vdots & \vdots & \cdots & \vdots & \cdots & \vdots & \cdots & \vdots & \Big\} \text{factor C} \\
 & Y_{11c1} & & Y_{11cn} & Y_{12c1} & & Y_{12cn} & & Y_{1bc1} & & Y_{1bcn} & \\
 & Y_{2111} & & Y_{211n} & Y_{2211} & & Y_{221n} & & Y_{2b11} & & Y_{2b1n} & \\
2 & \vdots & \cdots & \vdots & \vdots & \cdots & \vdots & \cdots & \vdots & \cdots & \vdots & \\
 & Y_{21c1} & & Y_{21cn} & Y_{22c1} & & Y_{22cn} & & Y_{2bc1} & & Y_{2bcn} & \\
\vdots & & \vdots & & & \vdots & & \vdots & & \vdots & & \\
 & Y_{a111} & & Y_{a11n} & Y_{a211} & & Y_{a21n} & & Y_{ab11} & & Y_{ab1n} & \\
a & \vdots & \cdots & \vdots & \vdots & \cdots & \vdots & \cdots & \vdots & \cdots & \vdots & \\
 & Y_{a1c1} & & Y_{a1cn} & Y_{a2c1} & & Y_{a2cn} & & Y_{abc1} & & Y_{abcn} & \\
\uparrow & & & & & & & & & & & \\
\text{factor A} & & & & & & & & & \underbrace{\qquad\qquad}_{\text{n replications}} & & \\
\end{array}
$$

Here, each Y_{ijkm} represents an observation at the same voxel across different *AFNI* 3D datasets.

We will consider 5 different three factor models, summarized in the following table:

Table1. Three Factor ANOVA Models

Model	Factor A	Factor B	Factor C	Structure
1	fixed	fixed	fixed	$A \times B \times C$
2	random	random	random	$A \times B \times C$
3	fixed	random	random	$A \times B \times C$
4	fixed	fixed	random	$A \times B \times C$
5	fixed	fixed	random	$A \times B, B \times C$ C nested in A

Models 1 through 4 are crossed models, i.e., each level of each factor appears with each level of every other factor. The crossed three factor ANOVA model is:

$$Y_{ijkm} = \mu_{...} + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkm},$$

where

$Y_{ijkm} =$ mth observation at level i of factor A, level j of factor B, and level k of factor C;
$\mu_{...} =$ constant;
α_i, β_j, γ_k, $(\alpha\beta)_{ij}$, $(\alpha\gamma)_{ik}$, $(\beta\gamma)_{jk}$, and $(\alpha\beta\gamma)_{ijk}$ represent either fixed effects or random effects, depending on the type of ANOVA model (see Table 1);
$\varepsilon_{ijkm} =$ random error, iid $N(0, \sigma^2)$.

Model 5 is a crossed-nested model; i.e., the levels of factor C are nested within the levels of factor A. For Model 5, the levels for factors A and B are *fixed*, while the levels of factor C are *random*. This three factor crossed-nested ANOVA model is given by:

$$Y_{ijkm} = \mu_{...} + \alpha_i + \beta_j + \gamma_{k(i)} + (\alpha\beta)_{ij} + (\beta\gamma)_{jk(i)} + \varepsilon_{m(ijk)},$$

where

$Y_{ijkm} =$ mth observation at level i of factor A, level j of factor B, and level k of factor C;
$\mu_{...} =$ constant;
α_i, β_j, and $(\alpha\beta)_{ij}$ represent fixed effects;
$\gamma_{k(i)}$ and $(\beta\gamma)_{jk(i)}$ represent random effects;
$\varepsilon_{m(ijk)} =$ random error, iid $N(0, \sigma^2)$.

The sums of squares for each of the above models are (where, as usual, a '.' in place of a particular subscript represents summation over the corresponding index):

$$SS0 = \frac{Y_{....}^2}{abcn}, \qquad SSI = \frac{\sum_i Y_{i...}^2}{bcn}, \qquad SSJ = \frac{\sum_j Y_{.j..}^2}{acn},$$

$$SSK = \frac{\sum_k Y_{..k.}^2}{abn}, \qquad SSIJ = \frac{\sum_i \sum_j Y_{ij..}^2}{cn}, \qquad SSIK = \frac{\sum_i \sum_k Y_{i.k.}^2}{bn},$$

$$SSJK = \frac{\sum_j \sum_k Y_{.jk.}^2}{an}, \quad SSIJK = \frac{\sum_i \sum_j \sum_k Y_{ijk.}^2}{n}, \quad SSIJKM = \sum_i \sum_j \sum_k \sum_m Y_{ijkm}^2,$$

$$\begin{aligned} SSTO &= SSIJKM - SS0, \\ SSE &= SSTO - SSIJK + SS0, \end{aligned}$$

$$
\begin{aligned}
SSA &= SSI - SS0, \\
SSB &= SSJ - SS0, \\
SSC &= SSK - SS0, \\
SSAB &= SSIJ - SSA - SSB - SS0, \\
SSAC &= SSIK - SSA - SSC - SS0, \\
SSBC &= SSJK - SSB - SSC - SS0, \\
\text{and } SSABC &= SSTO - SSE - SSA - SSB - SSC \\
&\quad -SSAB - SSAC - SSBC.
\end{aligned}
$$

For Model 5 (factor C nested in factor A), we also have:

$$
\begin{aligned}
SSC(A) &= SSIK - SSA - SS0, \\
SSBC(A) &= SSTO - SSE - SSA - SSB \\
&\quad -SSC(A) - SSAB.
\end{aligned}
$$

The mean squares are obtained by dividing the sum of squares by the corresponding degrees of freedom. The above can be summarized in an ANOVA table for Models 1 through 4:

Table 2. ANOVA Table for (Crossed) Three Factor Study

Source of Variation	SS	df	MS
Factor A	SSA	$a-1$	$MSA = \frac{SSA}{a-1}$
Factor B	SSB	$b-1$	$MSB = \frac{SSB}{b-1}$
Factor C	SSC	$c-1$	$MSC = \frac{SSC}{c-1}$
A×B Interaction	$SSAB$	$(a-1)(b-1)$	$MSAB = \frac{SSAB}{(a-1)(b-1)}$
A×C Interaction	$SSAC$	$(a-1)(c-1)$	$MSAC = \frac{SSAC}{(a-1)(c-1)}$
B×C Interaction	$SSBC$	$(b-1)(c-1)$	$MSBC = \frac{SSBC}{(b-1)(c-1)}$
A×B×C Interaction	$SSABC$	$(a-1)(b-1)(c-1)$	$MSABC = \frac{SSABC}{(a-1)(b-1)(c-1)}$
Error	SSE	$abc(n-1)$	$MSE = \frac{SSE}{abc(n-1)}$
Total	$SSTO$	$abcn-1$	

and, for Model 5, we have the following ANOVA table:

Table 3. ANOVA Table for Crossed-Nested Three Factor Study

Source of Variation	SS	df	MS
Factor A	SSA	$a-1$	$MSA = \frac{SSA}{a-1}$
Factor B	SSB	$b-1$	$MSB = \frac{SSB}{b-1}$
Factor C(A)	$SSC(A)$	$a(c-1)$	$MSC(A) = \frac{SSC(A)}{a(c-1)}$
A×B Interaction	$SSAB$	$(a-1)(b-1)$	$MSAB = \frac{SSAB}{(a-1)(b-1)}$
B×C(A) Interaction	$SSBC(A)$	$a(b-1)(c-1)$	$MSBC(A) = \frac{SSBC(A)}{a(b-1)(c-1)}$
Error	SSE	$abc(n-1)$	$MSE = \frac{SSE}{abc(n-1)}$
Total	$SSTO$	$abcn-1$	

3.2.2 F-tests

The above models differ in the appropriate choice for the test statistics. Table 4 summarizes the test statistics which are used depending on the model type, for testing the different factor main effects and factor interactions.

Table 4. Test Statistics

Model	$F(A)$	$F(B)$	$F(C)$	$F(AB)$	$F(AC)$	$F(BC)$	$F(ABC)$
1	$\frac{MSA}{MSE}$	$\frac{MSB}{MSE}$	$\frac{MSC}{MSE}$	$\frac{MSAB}{MSE}$	$\frac{MSAC}{MSE}$	$\frac{MSBC}{MSE}$	$\frac{MSABC}{MSE}$
2	–	–	–	$\frac{MSAB}{MSABC}$	$\frac{MSAC}{MSABC}$	$\frac{MSBC}{MSABC}$	$\frac{MSABC}{MSE}$
3	–	$\frac{MSB}{MSBC}$	$\frac{MSC}{MSBC}$	$\frac{MSAB}{MSABC}$	$\frac{MSAC}{MSABC}$	$\frac{MSBC}{MSE}$	$\frac{MSABC}{MSE}$
4	$\frac{MSA}{MSAC}$	$\frac{MSB}{MSBC}$	$\frac{MSC}{MSE}$	$\frac{MSAB}{MSABC}$	$\frac{MSAC}{MSE}$	$\frac{MSBC}{MSE}$	$\frac{MSABC}{MSE}$
5	$\frac{MSA}{MSC(A)}$	$\frac{MSB}{MSBC(A)}$	$\frac{MSC(A)}{MSE}$	$\frac{MSAB}{MSBC(A)}$	–	$\frac{MSBC(A)}{MSE}$	–

Missing entries in the table are due to either the absence of an exact F-test for those particular cases (e.g., there is no exact F-test for the presence of factor A main effect for Model 2), or the particular F-test in question is meaningless (as would be an F-test for interaction of factors A and C for Model 5, where factor C is nested in factor A). Note that the presence of an entry in the above table does not guarantee that the corresponding F-statistic can be calculated for a particular problem. Specifically, if there is only one sample per cell for a particular experiment ($n = 1$), then there are zero degrees of freedom for estimating MSE (i.e., $MSE = 0$). Obviously, no F-test can be performed which would require computing an F-statistic where this MSE appears in the denominator.

3.2.3 Estimation of factor level means, differences, and contrasts

The procedure for estimation of factor levels means, differences in factor level means, and contrasts in factor level means, is similar to that as described for programs 3dANOVA and 3dANOVA2. Calculation of the t-test statistics is also similar. The general rule is as follows: to calculate the estimated variance for a fixed factor level, use the denominator of the corresponding F-statistic from Table 4. For example, suppose that Model 5 is being used, and that one wishes to estimate the difference between levels 3 and 4 of factor B. This difference is estimated by:

$$\hat{D}_{3,4} = \bar{Y}_{.3..} - \bar{Y}_{.4..}$$

Now, from Table 4, we see that, for Model 5, $F(B) = \frac{MSB}{MSBC(A)}$. Hence, the mean sum of squares to use is $MSBC(A)$. Therefore, the t-statistic for testing whether levels 3 and 4 of factor B are significantly different is:

$$t^* = \frac{\hat{D}_{3,4}}{\sqrt{MSBC(A)\left(\frac{2}{acn}\right)}}$$

Similarly for calculation of contrasts of fixed factor level means.

3.2.4 Simultaneous inference

The discussion for program 3dANOVA concerning the appropriate confidence levels when multiple comparisons are made applies here as well.

3.3 Usage

The command line format for program 3dANOVA3 is as follows:

3dANOVA3 \
–**type** *k* **-alevels** *a* **-blevels** *b* **-clevels** *c* \
-dset *1 1 1 filename* ... **-dset** *1 1 1 filename* \
⋮
-dset *a b c filename* ... **-dset** *a b c filename* \
[**-voxel** *num*] \
[**-diskspace**] \
[**-fa** *prefixname*] [**-fb** *prefixname*] [**-fc** *prefixname*] \
[**-fab** *prefixname*] [**-fac** *prefixname*] [**-fbc** *prefixname*] [**-fabc** *prefixname*] \
[**-amean** *i prefixname*] [**-bmean** *j prefixname*] [**-cmean** *k prefixname*] \
[**-xmean** *i j k prefixname*] \
[**-adiff** *i j prefixname*] [**-bdiff** *i j prefixname*] [**-cdiff** *i j prefixname*] \
[**-xdiff** *i j k l m n prefixname*] \
[**-acontr** $c_1 \ldots c_a$ *prefixname*] [**-bcontr** $c_1 \ldots c_b$ *prefixname*] \
[**-ccontr** $c_1 \ldots c_c$ *prefixname*] \
[**-bucket** *prefixname*]

3.4 Options:

-type *k*
The -type command is used to identify which model to use in the analysis. The integer k must correspond to one of the 5 models listed in Table 1.

-alevels *a*
The -alevels command tells program 3dANOVA3 that a levels will be used for factor A. The number a following -alevels must be an integer, $1 < a \leq 100$. The -alevels command is mandatory, and must appear before any -dset command.

-blevels *b*
The -blevels command indicates that b levels will be used for factor B. The number b following -blevels must be an integer, $1 < b \leq 100$. The -blevels command is mandatory, and must appear before any -dset command.

-clevels *c*
The -clevels command indicates that c levels will be used for factor C. The number c following -clevels must be an integer, $1 < c \leq 100$. The -clevels command is mandatory, and must appear before any -dset command.

-dset *i j k filename*
The -dset command is used to tell program 3dANOVA3 which datasets to use in the analysis. The first number, i, which follows -dset specifies the level of factor A for this dataset; the second number, j, specifies the level of factor B; and the third number, k, specifies the level of factor C. The last item, "filename", is the name of an *AFNI* 3D dataset. Both the ".HEAD" and ".BRIK" files must be available. Obviously, the integers i, j, and k must satisfy $1 \leq i \leq a$,$1 \leq j \leq b$, and $1 \leq k \leq c$, where $a =$ number of levels of factor A, $b =$ number of levels of factor B, and $c =$ number of levels of factor C.

There must be a separate -dset command for each data set in the analysis. The data sets may be entered in any order. It is required that there be an equal number of data sets for each combination of levels for factors A, B, and C. There is a limit of 100 replications per cell. Of course, the data sets must have identical dimensions.

-voxel *num*
The -voxel command instructs program 3dANOVA3 to display the intermediate ANOVA calculations for the single voxel *num*. This makes it possible to verify the ANOVA calculations for a particular voxel. Since program 3dANOVA3 takes a while to execute, the -voxel command is useful for reassuring the user that the program has not wandered away.

-diskspace
The -diskspace command tells program 3dANOVA3 to calculate how much free disk space is required to execute the given task. The amount of disk space required depends upon the dimensions of the input datasets (i.e., number of voxels per image), the particular ANOVA

model being used, and the number of output datasets requested. The program prints to the screen how much disk space is required, and a tabulation of the disk space currently available on the system. The program then asks the operator if he wishes to continue.

-fa ***prefixname***

The -fa command is used to test for factor A main effect. This command is available for Models 1, 4, and 5. Program 3dANOVA3 calculates an F-test for equality of level means for factor A. The output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to factor A effect ($\sqrt{MSA}$) for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$\textit{AFNI}\ \text{“fift” dataset} \qquad \left\{ \begin{array}{l} \boxed{I = \sqrt{MSA}} \\ \\ \boxed{F^* = F(A) \text{ from Table 4.}} \end{array} \right.$$

The calculated F-statistic corresponds to the model type, as listed in Table 4 under $F(A)$. The numerator and denominator degrees of freedom for the F-statistic (from Table 2 or 3) are contained in the ".HEAD" file for this dataset.

-fb ***prefixname***

The -fb command is used to test for factor B main effect. This command is available for Models 1, 3, 4, and 5. Program 3dANOVA3 calculates an F-test for equality of level means for factor B. The output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to factor B effect ($\sqrt{MSB}$) for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$\textit{AFNI}\ \text{”fift” dataset} \qquad \left\{ \begin{array}{l} \boxed{I = \sqrt{MSB}} \\ \\ \boxed{F^* = F(B) \text{ from Table 4.}} \end{array} \right.$$

The calculated F-statistic corresponds to the model type, as listed in Table 4 under $F(B)$. The numerator and denominator degrees of freedom for the F-statistic (from Table 2 or 3) are contained in the ".HEAD" file for this dataset.

-fc ***prefixname***

The -fc command is used to test for factor C main effect. This command is available for Models 1, 3, 4, and 5. Program 3dANOVA3 calculates an F-test for equality of level means for factor C. The output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to factor C effect for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$\textit{AFNI}\ \text{”fift” dataset} \qquad \left\{ \begin{array}{l} \boxed{I = \left\{ \begin{array}{ll} \sqrt{MSC} & \text{Models 1, 3, 4} \\ \\ \sqrt{MSC(A)} & \text{Model 5} \end{array} \right.} \\ \\ \boxed{F^* = F(C) \text{ from Table 4.}} \end{array} \right.$$

The calculated F-statistic corresponds to the model type, as listed in Table 4 under $F(C)$. The numerator and denominator degrees of freedom for the F-statistic (from Table 2 or 3) are contained in the ".HEAD" file for this dataset.

-fab *prefixname*

The -fab command is used to test for the presence of interaction effect of factors A and B. This command is available for all models. Program 3dANOVA3 calculates an F-test for AB interaction . The output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to interaction ($\sqrt{MSAB}$) for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$\textit{AFNI}\ \text{“fift” dataset} \quad \left\{ \begin{array}{l} \boxed{I = \sqrt{MSAB}} \\ \\ \boxed{F^* = F(AB) \text{ from Table 4.}} \end{array} \right.$$

The calculated F-statistic corresponds to the model type, as listed in Table 4 under $F(AB)$. The numerator and denominator degrees of freedom for the F-statistic (from Table 2 or 3) are contained in the ".HEAD" file for this dataset.

-fac *prefixname*

The -fac command is used to test for the presence of interaction effect of factors A and C. This command is available for models 1, 2, 3, and 4. Program 3dANOVA3 calculates an F-test for AC interaction. The output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to interaction ($\sqrt{MSAC}$) for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$\textit{AFNI}\ \text{“fift” dataset} \quad \left\{ \begin{array}{l} \boxed{I = \sqrt{MSAC}} \\ \\ \boxed{F^* = F(AC) \text{ from Table 4.}} \end{array} \right.$$

The calculated F-statistic corresponds to the model type, as listed in Table 4 under $F(AC)$. The numerator and denominator degrees of freedom for the F-statistic (from Table 2 or 3) are contained in the ".HEAD" file for this dataset.

-fbc *prefixname*

The -fbc command is used to test for the presence of interaction effect of factors B and C. This command is available for all models. Program 3dANOVA3 calculates an F-test for BC interaction. The output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to interaction for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$
AFNI \text{ ``fift'' dataset} \quad \begin{cases} \boxed{I = \begin{cases} \sqrt{MSBC} & \text{Models 1, 2, 3, 4} \\ \\ \sqrt{MSBC(A)} & \text{Model 5} \end{cases}} \\ \\ \boxed{F^* = F(BC) \text{ from Table 4.}} \end{cases}
$$

The calculated F-statistic corresponds to the model type, as listed in Table 4 under $F(BC)$. The numerator and denominator degrees of freedom for the F-statistic (from Table 2 or 3) are contained in the ".HEAD" file for this dataset.

-fabc *prefixname*

The -fabc command is used to test for the presence of interaction effect of factors A, B, and C. This command is available for models 1, 2, 3, and 4. Program 3dANOVA3 calculates an F-test for ABC interaction. The output consists of a 2 sub-brick *AFNI* dataset of type "fift". The first sub-brick consists of the square root of the mean sum of squares due to interaction for each voxel, and the second sub-brick contains the corresponding F-statistics.

$$
AFNI \text{ ``fift'' dataset} \quad \begin{cases} \boxed{I = \sqrt{MSABC}} \\ \\ \boxed{F^* = F(ABC) \text{ from Table 4.}} \end{cases}
$$

The numerator and denominator degrees of freedom for the F-statistic (from Table 2) are contained in the ".HEAD" file for this dataset.

-amean *i prefixname*

The -amean command is used to estimate the mean of level i for factor A. This command is available for Models 1, 4, and 5. Program 3dANOVA3 calculates the mean and the corresponding t-statistic of the mean for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt" in a file with the user specified prefix filename. The first sub-brick consists of the estimated mean $\bar{Y}_{i...}$, and the second sub-brick contains the corresponding t-statistic t^* for each voxel.

$$
AFNI \text{ ``fitt'' dataset} \quad \begin{cases} \boxed{\bar{Y}_{i...} = \dfrac{\sum_{j=1}^{b} \sum_{k=1}^{c} \sum_{m=1}^{n} Y_{ijkm}}{bcn}} \\ \\ \boxed{t^* = \dfrac{\bar{Y}_{i...}}{\sqrt{\dfrac{MS}{bcn}}}} \end{cases}
$$

Here MS represents the denominator of the test statistic $F(A)$ from Table 4 corresponding to the model being used.

-bmean *j prefixname*

The -bmean command is used to estimate the mean of level j for factor B. This command is available for Models 1, 4, and 5. Program 3dANOVA3 calculates the mean and the corresponding t-statistic of the mean for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt" in a file with the user specified prefix filename. The first sub-brick consists of the estimated mean $\bar{Y}_{.j..}$, and the second sub-brick contains the corresponding t-statistic t^* for each voxel.

$$\textit{AFNI}\text{ ``fitt'' dataset} \quad \left\{ \begin{array}{l} \boxed{\bar{Y}_{.j..} = \dfrac{\sum_{i=1}^{a}\sum_{k=1}^{c}\sum_{m=1}^{n} Y_{ijkm}}{acn}} \\ \\ \boxed{t^* = \dfrac{\bar{Y}_{.j..}}{\sqrt{\dfrac{MS}{acn}}}} \end{array} \right.$$

Here MS represents the denominator of the test statistic $F(B)$ from Table 4 corresponding to the model being used.

-cmean *k prefixname*

The -cmean command is used to estimate the mean of level k for factor C. This command is available for Model 1 only. Program 3dANOVA3 calculates the mean and the corresponding t-statistic of the mean for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt" in a file with the user specified prefix filename. The first sub-brick consists of the estimated mean $\bar{Y}_{..k.}$, and the second sub-brick contains the corresponding t-statistic t^* for each voxel.

$$\textit{AFNI}\text{ ``fitt'' dataset} \quad \left\{ \begin{array}{l} \boxed{\bar{Y}_{..k.} = \dfrac{\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{m=1}^{n} Y_{ijkm}}{abn}} \\ \\ \boxed{t^* = \dfrac{\bar{Y}_{..k.}}{\sqrt{\dfrac{MSE}{abn}}}} \end{array} \right.$$

-xmean *i j k prefixname*

The -xmean command is used to estimate the mean of the cell at level i of factor A, level j of factor B, and level k for factor C. This command is available for Model 1 only. Program 3dANOVA3 calculates the mean and the corresponding t-statistic of the mean for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt" in a file with the user specified prefix filename. The first sub-brick consists of the estimated mean $\bar{Y}_{ijk.}$, and the second sub-brick contains the corresponding t-statistic t^* for each voxel.

$$
AFNI \text{ “fitt” dataset} \quad \left\{ \begin{array}{l} \boxed{\bar{Y}_{ijk.} = \dfrac{\sum_{m=1}^{n} Y_{ijkm}}{n}} \\ \\ \boxed{t^* = \dfrac{\bar{Y}_{ijk.}}{\sqrt{\dfrac{MSE}{n}}}} \end{array} \right.
$$

-adiff ***i j prefixname***

The -adiff command is used to estimate the difference between two level means of factor A. This command is available for Models 1, 4, and 5. Program 3dANOVA3 calculates the difference in the estimated factor A means at levels i and j, and the corresponding t-statistic of the difference, for each voxel. The result is stored as a 2 sub-brick “fitt” *AFNI* dataset under the user specified prefix filename. The first sub-brick contains the estimated differences $\hat{D}_{i,j} = \bar{Y}_{i...} - \bar{Y}_{j...}$, and the second sub-brick contains the corresponding t-statistics.

$$
AFNI \text{ ”fitt” dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{D}_{i,j} = \bar{Y}_{i...} - \bar{Y}_{j...}} \\ \\ \boxed{t^* = \dfrac{\hat{D}_{i,j}}{\sqrt{MS\left(\dfrac{2}{bcn}\right)}}} \end{array} \right.
$$

Here MS represents the denominator of the test statistic $F(A)$ from Table 4 corresponding to the model being used.

-bdiff ***i j prefixname***

The -bdiff command is used to estimate the difference between two level means of factor B. This command is available for Models 1, 4, and 5. Program 3dANOVA3 calculates the difference in the estimated factor B means at levels i and j, and the corresponding t-statistic of the difference, for each voxel. The result is stored as a 2 sub-brick ”fitt” *AFNI* dataset under the user specified prefix filename. The first sub-brick contains the estimated differences $\hat{D}_{i,j} = \bar{Y}_{.i..} - \bar{Y}_{.j..}$, and the second sub-brick contains the corresponding t-statistics.

$$
AFNI \text{ “fitt” dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{D}_{i,j} = \bar{Y}_{.i..} - \bar{Y}_{.j..}} \\ \\ \boxed{t^* = \dfrac{\hat{D}_{i,j}}{\sqrt{MS\left(\dfrac{2}{acn}\right)}}} \end{array} \right.
$$

Here MS represents the denominator of the test statistic $F(B)$ from Table 4 corresponding to the model being used.

-cdiff ***i j prefixname***

The -cdiff command is used to estimate the difference between two level means of factor C. This command is available for Model 1 only. Program 3dANOVA3 calculates the difference in the estimated factor C means at levels i and j, and the corresponding t-statistic of the difference, for each voxel. The result is stored as a 2 sub-brick "fitt" *AFNI* dataset under the user specified prefix filename. The first sub-brick contains the estimated differences $\hat{D}_{i,j} = \bar{Y}_{..i.} - \bar{Y}_{..j.}$, and the second sub-brick contains the corresponding t-statistics.

$$AFNI \text{ “fitt” dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{D}_{i,j} = \bar{Y}_{..i.} - \bar{Y}_{..j.}} \\ \\ \boxed{t^* = \dfrac{\hat{D}_{i,j}}{\sqrt{MSE\left(\dfrac{2}{abn}\right)}}} \end{array} \right.$$

-xdiff ***i j k l m n prefixname***

The -xdiff command is used to estimate the difference between the means of two cells. This command is available for Model 1 only. Program 3dANOVA3 calculates the difference between the mean of the cell at $(A, B, C) = (i, j, k)$, and the mean of the cell at $(A, B, C) = (l, m, n)$, along with the corresponding t-statistic of the difference, for each voxel. The result is stored as a 2 sub-brick "fitt" *AFNI* dataset under the user specified prefix filename. The first sub-brick contains the estimated differences $\hat{D}_{i,j,k,l,m,n} = \bar{Y}_{ijk.} - \bar{Y}_{lmn.}$, and the second sub-brick contains the corresponding t-statistics.

$$AFNI \text{ “fitt” dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{D}_{i,j,k,l,m,n} = \bar{Y}_{ijk.} - \bar{Y}_{lmn.}} \\ \\ \boxed{t^* = \dfrac{\hat{D}_{i,j,k,l,m,n}}{\sqrt{MSE\left(\dfrac{2}{n}\right)}}} \end{array} \right.$$

-acontr $c_1 ... c_a$ ***prefixname***

The -acontr command is used to estimate a contrast in levels of factor A. This command is available for Models 1, 4, and 5. Program 3dANOVA3 calculates the estimated contrast $\hat{L} = \sum_{i=1}^{a} c_i \bar{Y}_{i...}$, along with the corresponding t-statistic, for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt", under the user specified prefix filename.

$$AFNI \text{ “fitt” dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{L} = \sum_{i=1}^{a} c_i \bar{Y}_{i...}} \\ \\ \boxed{t^* = \left\{ \dfrac{\hat{L}}{\sqrt{MS\left(\dfrac{1}{bcn}\right)\left(\sum_{i=1}^{a} c_i^2\right)}} \right.} \end{array} \right.$$

Here MS represents the denominator of the test statistic $F(A)$ from Table 4 corresponding to the model being used.

-bcontr $c_1 ... c_b$ ***prefixname***

The -bcontr command is used to estimate a contrast in levels of factor B. This command is available for Models 1, 4, and 5.Program 3dANOVA3 calculates the estimated contrast $\hat{L} = \sum_{j=1}^{b} c_j \bar{Y}_{.j..}$, along with the corresponding t-statistic, for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt", under the user specified prefix filename.

$$\textit{AFNI}\ \text{"fitt" dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{L} = \sum_{j=1}^{b} c_j \bar{Y}_{.j..}} \\ \\ \boxed{t^* = \dfrac{\hat{L}}{\sqrt{MS\left(\frac{1}{acn}\right)\left(\sum_{j=1}^{b} c_j^2\right)}}} \end{array} \right.$$

Here MS represents the denominator of the test statistic $F(B)$ from Table 4 corresponding to the model being used.

-ccontr $c_1 ... c_b$ ***prefixname***

The -ccontr command is used to estimate a contrast in levels of factor C. This command is available for Model 1 only. Program 3dANOVA3 calculates the estimated contrast $\hat{L} = \sum_{k=1}^{c} c_k \bar{Y}_{..k.}$, along with the corresponding t-statistic, for each voxel. The result is stored as a 2 sub-brick *AFNI* data set of type "fitt", under the user specified prefix filename.

$$\textit{AFNI}\ \text{"fitt" dataset} \quad \left\{ \begin{array}{l} \boxed{\hat{L} = \sum_{k=1}^{c} c_k \bar{Y}_{..k.}} \\ \\ \boxed{t^* = \dfrac{\hat{L}}{\sqrt{MSE\left(\frac{1}{abn}\right)\left(\sum_{k=1}^{c} c_k^2\right)}}} \end{array} \right.$$

-bucket ***prefixname***

The -bucket command is used in conjunction with the previously described output commands. All of the *AFNI* 2 sub-brick datasets, which are created with the above commands, are replaced with a single *AFNI* "bucket" type dataset. The labels for the individual sub-bricks within the bucket dataset are derived from the user specified prefix filenames from the corresponding output commands. The individual sub-bricks can be accessed for display within program afni. The purpose of this command is to simplify file management, since all of the output files for a particular problem are now contained within the single *AFNI* bucket dataset. (See Examples 1 and 2 for illustrations of the use of this command.)

Note that this command requires (the user's selection of) some of the output commands (-fa, -fb, -fc, -fab, -fac, -fbc, -fabc, -amean, -bmean, -cmean, -xmean, -adiff, -bdiff, -cdiff, -xdiff, -acontr, -bcontr, -ccontr) in order to determine which results to calculate, and what labels to apply to the individual sub-bricks.

3.5 Notes

- Since program 3dANOVA3 uses temporary disk files to hold the results of intermediate calculations, the user should be aware that there must be enough available disk space for the temporary files, in addition to the permanent output files. If the -diskspace command is used, then prior to the start of the ANOVA calculations, program 3dANOVA3 calculates the maximum amount of disk space required to solve the given problem.
- The program will not overwrite previously existing files. Therefore, if a file already exists with the same name as an output file specified by the user, the program will terminate. This applies as well to the temporary data files created by the program. All temporary files created by program 3dANOVA3 have the suffix ".3danova3". If, for any reason, the program should prematurely terminate execution, the user must manually remove any ".3danova3" files that may be left.
- This program cannot handle complex-valued datasets or time-dependent datasets.

3.6 Examples

Example 1. A researcher wishes to study differences in neural activation arising from 3 factors: factor A: sex (level 1 = Male, level 2 = Female), factor B: handedness (level 1 = Left, level 2 = Right), and factor C: smoking (level 1 = non-smoking, level 2 = smoking). There were 3 subjects for each combination of factor levels. A batch command file to study the results is presented below.

3dANOVA3 Batch Command File for Example 1

```
/usr2/ward/AFNI96/3dANOVA3 \
-type 1 \
-alevels 2 \
-blevels 2 \
-clevels 2 \
-dset 1 1 1 subj01.male.left.nonsm+tlrc \
-dset 1 1 1 subj02.male.left.nonsm+tlrc \
-dset 1 1 1 subj03.male.left.nonsm+tlrc \
-dset 1 1 2 subj04.male.left.smoke+tlrc \
-dset 1 1 2 subj05.male.left.smoke+tlrc \
-dset 1 1 2 subj06.male.left.smoke+tlrc \
-dset 1 2 1 subj07.male.right.nonsm+tlrc \
-dset 1 2 1 subj08.male.right.nonsm+tlrc \
-dset 1 2 1 subj09.male.right.nonsm+tlrc \
-dset 1 2 2 subj10.male.right.smoke+tlrc \
-dset 1 2 2 subj11.male.right.smoke+tlrc \
-dset 1 2 2 subj12.male.right.smoke+tlrc \
```

```
-dset 2 1 1 subj13.female.left.nonsm+tlrc \
-dset 2 1 1 subj14.female.left.nonsm+tlrc \
-dset 2 1 1 subj15.female.left.nonsm+tlrc \
-dset 2 1 2 subj16.female.left.smoke+tlrc \
-dset 2 1 2 subj17.female.left.smoke+tlrc \
-dset 2 1 2 subj18.female.left.smoke+tlrc \
-dset 2 2 1 subj19.female.right.nonsm+tlrc \
-dset 2 2 1 subj20.female.right.nonsm+tlrc \
-dset 2 2 1 subj21.female.right.nonsm+tlrc \
-dset 2 2 2 subj22.female.right.smoke+tlrc \
-dset 2 2 2 subj23.female.right.smoke+tlrc \
-dset 2 2 2 subj24.female.right.smoke+tlrc \
-voxel 1234567 \
-diskspace \
-fa FactorA \
-fb FactorB \
-fc FactorC \
-fab AxB\
-fac AxC \
-fbc BxC \
-fabc AxBxC
```

■

It is obvious that this is a three-factor study, with all factors fixed (i.e., each factor is of intrinsic interest). Therefore, the command -type 1 is used, indicating that Model 1 is appropriate.

There were 3 subjects for each combination of factor levels, for a total of $2 \times 2 \times 2 \times 3 = 24$ datasets. Therefore, there are 24 separate -dset i j k filename commands, where i = level of factor A, j = level of factor B, and k = level of factor C for that particular dataset.

Since the operator has used the -voxel command, the ANOVA calculations for that particular voxel are written to the screen.

The -diskspace command means that the program is to display the disk space required for program execution, prior to the start of the actual calculations.

Tests for significance of factor main effects and factor interactions are produced by the -fa, -fb, -fc, -fab, -fac, -fbc, and -fabc commands. The resulting *AFNI* 'fift' datasets have the user specified prefix filenames.

The program output which is sent to the screen is as follows:

3dANOVA3 Screen Output for Example 1

```
Program 3dANOVA3

Last revision: 28 January 1997
Data set dimensions: nx = 161 ny = 191 nz = 141 nxyz = 4335891
```

Number of input datasets = 24

This problem requires approximately 261 MB of free disk space.
Summary of available disk space:

Filesystem	Type	kbytes	use	avail	%use	Mounted on
/dev/dsk/dks1d4s7	xfs	8868612	479440	8389172	6	/usr2

Do you wish to proceed? y

y[1][1][1][1] = 284.000000
y[1][1][1][2] = 224.000000
y[1][1][1][3] = 52.000000
y[1][1][2][1] = 199.000000
y[1][1][2][2] = 578.000000
y[1][1][2][3] = 240.000000
⋮
y[2][2][2][1] = 689.000000
y[2][2][2][2] = 751.000000
y[2][2][2][3] = 946.000000
y[2][2][2][.] = 2386.000000
SSIJK = 7237407.000000
SSIJKM = 8452865.000000
SSTO = 2304964.500000
SSE = 1215458.000000
SSA = 111656.500000
SSB = 405860.000000
SSC = 387858.000000
SSAB = 48691.000000
SSAC = 97410.500000
SSBC = 27405.000000
SSABC = 10625.500000
MSA = 111656.500000
F(A) = 1.469820
— Writing combined dataset into ./FactorA+tlrc.HEAD
MSB = 405860.000000
F(B) = 5.342645
— Writing combined dataset into ./FactorB+tlrc.HEAD
MSC = 387858.000000
F(C) = 5.105670
— Writing combined dataset into ./FactorC+tlrc.HEAD
MSAB = 48691.000000
F(AB) = 0.640957
— Writing combined dataset into ./AxB+tlrc.HEAD
MSAC = 97410.500000

F(AC) = 1.282289
— Writing combined dataset into ./AxC+tlrc.HEAD
MSBC = 27405.000000
F(BC) = 0.360753
— Writing combined dataset into ./BxC+tlrc.HEAD
MSABC = 10625.500000
F(ABC) = 0.139872
— Writing combined dataset into ./AxBxC+tlrc.HEAD

■

The program began by informing the user that 261 MB of free disk space are required for program execution (of which 121 MB are required for the 7 permanent *AFNI* 'fift' datasets). The program then displayed the currently available disk space, and asked if the operator wished to continue. After the operator responded with a 'y' (for 'yes'), the program displayed the ANOVA calculations as they were performed for voxel #1234567 (as specified by the -voxel command). Output of the form $y[i][j][k][m] = n$, where i, j, k,and m are integers, means that the intensity for voxel #1234567 of the mth dataset for level i of factor A, level j of factor B, and level k of factor C, is equal to n. As always, a '.' in place of a particular index means summation over all values for that index; e.g.,

$$y[2][2][2][.] = y[2][2][2][1] + y[2][2][2][2] + y[2][2][2][3]$$

The various sums of squares calculated by the program for voxel #1234567 were printed out, followed by the requested F-statistics.

Using the above results for voxel #1234567, one could construct the following ANOVA table:

ANOVA Table for Example 1, Voxel #1234567

Source of Variation	SS	df	MS	F*
Factor A	111656	1	111656	1.47
Factor B	405860	1	405860	5.34
Factor C	387858	1	387858	5.11
A×B Interaction	48691	1	48691	0.64
A×C Interaction	97410	1	97410	1.28
B×C Interaction	27405	1	27405	0.36
A×B×C Interaction	10625	1	10625	0.14
Error	1215458	16	(75966)	
Total	2304964	23		

As mentioned above, a total of 7 separate *AFNI* 3d datasets (14 files in all) were created by execution of this batch command file. To simplify file management, all of these datasets can be grouped into a single AFNI "bucket" dataset by appending the -bucket command, as shown below.

3dANOVA3 Batch Command File for Example 1

```
/usr2/ward/AFNI96/3dANOVA3 \
-type 1 \
-alevels 2 \
-blevels 2 \
-clevels 2 \
        ⋮
(same as above)
        ⋮
-fa FactorA \
-fb FactorB \
-fc FactorC \
-fab AxB\
-fac AxC \
-fbc BxC \
-fabc AxBxC \
-bucket test.results
```

■

The calculations performed are exactly the same as above. However, all of the output results are now stored in the *AFNI* "bucket" dataset "test.results" (.HEAD and .BRIK). The contents of the sub-bricks within the dataset are indicated by their labels:

Brick	Label	Contents
#0	FactorA:Inten	$I = \sqrt{MSA}$
#1	FactorA:F-stat	$F(A)$
#2	FactorB:Inten	$I = \sqrt{MSB}$
#3	FactorB:F-stat	$F(B)$
#4	FactorC:Inten	$I = \sqrt{MSC}$
#5	FactorC:F-stat	$F(C)$
#6	AxB:Inten	$I = \sqrt{MSAB}$
#7	AxB:F-stat	$F(AB)$
#8	AxC:Inten	$I = \sqrt{MSAC}$
#9	AxC:F-stat	$F(AC)$
#10	BxC:Inten	$I = \sqrt{MSBC}$
#11	BxC:F-stat	$F(BC)$
#12	AxBxC:Inten	$I = \sqrt{MSABC}$
#13	AxBxC:F-stat	$F(ABC)$

Note that the sub-bricks are stored in pairs. This follows the usual *AFNI* convention: the first sub-brick in each pair contains some computed quantity or estimate; the second sub-brick in each pair contains the corresponding statistic, which may be used as a display threshold within program afni. Also, note how the labels are derived from the corresponding output commands. These labels are used within afni to identify the individual sub-bricks.

Example 2.

In this example, the researcher is evaluating the differences in neural activation among 3 test procedures for Declarative memory. The different test procedures constitute the levels of factor A. Each test procedure is used to study Episodic and Semantic memory (factor B). Ten subjects were involved in the study, and each subject was studied under each test condition.

3dANOVA3 Batch Command File for Example 2

```
/usr2/ward/AFNI96/3dANOVA3 \
-type 4 \
-alevels 3 \
-blevels 2 \
-clevels 10 \
-dset 1 1 1 subj01.test1.epi+tlrc \
-dset 2 1 1 subj01.test2.epi+tlrc \
-dset 3 1 1 subj01.test3.epi+tlrc \
-dset 1 2 1 subj01.test1.sem+tlrc \
-dset 2 2 1 subj01.test2.sem+tlrc \
-dset 3 2 1 subj01.test3.sem+tlrc \
    ⋮
-dset 1 1 10 subj10.test1.epi+tlrc \
-dset 2 1 10 subj10.test2.epi+tlrc \
-dset 3 1 10 subj10.test3.epi+tlrc \
-dset 1 2 10 subj10.test1.sem+tlrc \
-dset 2 2 10 subj10.test2.sem+tlrc \
-dset 3 2 10 subj10.test3.sem+tlrc \
-voxel 1234567 \
-diskspace \
-fa Test \
-fb Memory \
-fab TxM \
-adiff 1 2 T1-T2 \
-adiff 1 3 T1-T3 \
-adiff 2 3 T2-T3 \
-bucket memory.test
```

■

Factors A and B are obviously fixed, whereas factor C is random (the subjects are not of intrinsic interest, but represent a random sample from the much larger population). The command -type 4 is used, as this experiment corresponds to Model 4 in Table 1.

In the above example, there are 3 levels for the factor A (different test procedures), and so the command -alevels 3 is used. That there are two types of memory under study is indicated by the command -blevels 2. And since there are 10 subjects (levels of factor C), the command -clevels 10 is used.

Since there are 10 data sets for each of the 3 levels of factor A and 2 levels of factor B, this requires $3 \times 2 \times 10 = 60$ separate -dset i j k filename commands.

The investigator has requested an F-test for the factor A (test procedure) effect by using the -fa command. The results are to be labeled "Test". An F-test for factor B (type of memory) effect is generated by the -fb command, and the results are labeled "Memory". Also, an F-test for the interaction effect between "procedure" and "type of memory" is produced by the -fab command. The results are labeled "TxM".

Also, the user requested 3 t-tests to examine the differences between the test procedures.

When the above batch file was executed, the screen output was as follows:

3dANOVA3 Screen Output for Example 2

Program 3dANOVA3

Last revision: 9 January 1998
Data set dimensions: nx = 161 ny = 191 nz = 141 nxyz = 4335891
Number of input datasets = 60

This problem requires approximately 226 MB of free disk space.
Summary of available disk space:

Filesystem	Type	kbytes	use	avail	%use	Mounted on
/dev/dsk/dks1d4s7	xfs	8868612	6694544	2174068	76	/usr2

Do you wish to proceed? y

y[1][1][1][1] = 384.000000
y[1][1][2][1] = 229.000000
y[1][1][3][1] = 702.000000
⋮
y[3][2][7][.] = 792.000000
y[3][2][8][.] = 336.000000
y[3][2][9][.] = 603.000000
y[3][2][10][.] = 749.000000
SSIJK = 21570300.000000
SSTO = 4059176.000000

SSA = 64504.000000
SSB = 16334.000000
SSC = 766634.000000
SSAB = 233020.000000
SSAC = 1385344.000000
SSBC = 508488.000000
SSABC = 1084852.000000
MSA = 32252.000000
F(A) = 0.419055
— Writing combined dataset into ./Test+tlrc.HEAD
MSB = 16334.000000
F(B) = 0.289104
— Writing combined dataset into ./Memory+tlrc.HEAD
MSAB = 116510.000000
F(AB) = 1.933149
— Writing combined dataset into ./TxM+tlrc.HEAD
⋮
Difference of factor A level 1 - level 2 = 74.400024
t for difference of factor A level 1 - level 2 = 0.848068
— Writing combined dataset into ./T1-T2+tlrc.HEAD
⋮
Difference of factor A level 1 - level 3 = 11.000000
t for difference of factor A level 1 - level 3 = 0.125386
— Writing combined dataset into ./T1-T3+tlrc.HEAD
⋮
Difference of factor A level 2 - level 3 = -63.400024
t for difference of factor A level 2 - level 3 = -0.722681
— Writing combined dataset into ./T2-T3+tlrc.HEAD
Writing 'bucket' dataset into memory.test
Processing dataset ./memory.test+tlrc.HEAD

■

We see that 226 MB of disk space were required for this specific problem (including 104 MB for the permanent output 'bucket' dataset "memory.test+tlrc" (.HEAD and .BRIK)). This is in addition to the 480 MB required to store the input data. The structure of the output bucket dataset is depicted below.

Brick	Label	Contents
#0	Test:Inten	$I = \sqrt{MSA}$
#1	Test:F-stat	$F(A)$
#2	Memory:Inten	$I = \sqrt{MSB}$
#3	Memory:F-stat	$F(B)$
#4	TxM:Inten	$I = \sqrt{MSAB}$
#5	TxM:F-stat	$F(AB)$
#6	T1-T2:Diff	$\hat{D}_{1,2} = \bar{Y}_{1\ldots} - \bar{Y}_{2\ldots}$
#7	T1–T2:t-stat	t-stat for $\hat{D}_{1,2}$
#8	T1-T3:Diff	$\hat{D}_{1,3} = \bar{Y}_{1\ldots} - \bar{Y}_{3\ldots}$
#9	T1-T3:t-stat	t-stat for $\hat{D}_{1,3}$
#10	T2-T3:Diff	$\hat{D}_{2,3} = \bar{Y}_{2\ldots} - \bar{Y}_{3\ldots}$
#11	T2-T3:t-stat	t-stat for $\hat{D}_{2,3}$

Besides the F-tests for factor A and factor B main effect, and $A \times B$ interaction, the program calculated the t-tests for significant differences in levels of factor A. To estimate the difference between levels 1 and 2 of factor A,

$$\hat{D}_{1,2} = \bar{Y}_{1\ldots} - \bar{Y}_{2\ldots} \ ,$$

which, from the screen output, is 74.40 (for voxel #1234567). From Table 4, we see that, for Model 4, $F(A) = \frac{MSA}{MSAC}$. Hence, the mean sum of squares to use in calculating the t-statistic is $MSAC$. Therefore, the t-statistic for testing whether levels 1 and 2 of factor A are significantly different is:

$$t^* = \frac{\hat{D}_{1,2}}{\sqrt{MSAC\left(\frac{2}{bcn}\right)}}$$

For voxel #1234567, $MSAC = \frac{SSAC}{(a-1)(c-1)} = 1385344/(2(9)) = 76964$. Therefore,

$$t^* = \frac{74.40}{\sqrt{76964\left(\frac{2}{2(10)(1)}\right)}} = 0.8481 \ .$$

Using the screen output for voxel #1234567, one could construct the following ANOVA table:

ANOVA Table for Example 2, Voxel #1234567

Source of Variation	SS	df	MS	F*
Factor A	64504	2	32252	0.42
Factor B	16334	1	16334	0.29
Factor C	766634	9	(85182)	—
A×B Interaction	233020	2	116510	1.93
A×C Interaction	1385344	18	(76964)	—
B×C Interaction	508488	9	(56499)	—
A×B×C Interaction	1084852	18	(60270)	—
Total	4059176	59		

Example 3. A researcher wishes to study differences in neural activation arising from the presence or absence of a particular disease. Group 1 consists of 15 persons diagnosed with this disease, and group 2 consists of 15 persons who do not have the disease. Each subject is given, on 5 consecutive days, a particular test. In this experiment, factor A is the presence or absence of the disease, and thus has 2 levels. Factor B represents the trial number for the test on consecutive days, so factor B has 5 levels. Factor C, the individual subject within a group, has 15 levels. To evaluate the results, program 3dANOVA3 was used, with the following batch command file.

3dANOVA3 Batch Command File for Example 3

```
/usr2/ward/AFNI96/3dANOVA3 \
-type 5 \
-alevels 2 \
-blevels 5 \
-clevels 15 \
-dset 1 1 01 group1.trial1.subj01+tlrc \
-dset 1 1 02 group1.trial1.subj02+tlrc \
-dset 1 1 03 group1.trial1.subj03+tlrc \
    ⋮
-dset 2 5 13 group2.trial5.subj28+tlrc \
-dset 2 5 14 group2.trial5.subj29+tlrc \
-dset 2 5 15 group2.trial5.subj30+tlrc \
-voxel 1234567 \
-diskspace \
-fa groupftest \
-fb trialftest \
-fab gtftest
```

■

In this example, both factor A and factor B are fixed, since the factor levels are of intrinsic interest. Factor C, the individual subject, is random. Also, factor C is *nested* within factor A; e.g., the ith subject for factor A level 1 (disease present) is different from the ith subject for factor A level 2 (disease absent). Therefore, the command -type 5 is used, indicating that Model 5 is appropriate.

In the above example, there are 2 levels for factor A (disease present and disease absent), and so the command -alevels 2 is used. That there are 5 consecutive trials is indicated by the command -blevels 5. And since there are 15 subjects (levels of factor C) for each level of factor A, the command -clevels 15 is used.

Since there are 15 data sets for each of the 2 levels of factor A and 5 levels of factor B, this requires $2 \times 5 \times 15 = 150$ separate -dset i j k filename commands, where i = level of factor A, j = level of factor B, and k = level of factor C.

The investigator has requested an F-test for the factor A (disease) effect by using the -fa command. The results are to be stored in file name "groupftest+tlrc" (.HEAD and .BRIK) in the current directory.

An F-test for factor B (trial) effect is generated by the -fb command. The results are to be stored in file name "trialftest+tlrc" (.HEAD and .BRIK) in the current directory. Also, an F-test for the interaction effect between "disease" and "trial" is produced by the -fab command. The results are to be stored in file name "gtftest+tlrc" (.HEAD and .BRIK) in the current directory.

When the above batch file was executed, the screen output was as follows:

3dANOVA3 Screen Output for Example 3

Program 3dANOVA3

Last revision: 28 January 1997
Data set dimensions: nx = 161 ny = 191 nz = 141 nxyz = 4335891
Number of input datasets = 150

This problem requires approximately 139 MB of free disk space.
Summary of available disk space:

Filesystem	Type	kbytes	use	avail	%use	Mounted on
/dev/dsk/dks1d4s7	xfs	8868612	1547432	7321180	18	/usr2

Do you wish to proceed? y

y[1][1][1][1] = 805.000000
y[1][1][2][1] = 319.000000
y[1][1][3][1] = 833.000000
y[1][1][4][1] = 347.000000
⋮

y[2][5][12][.] = 569.000000
y[2][5][13][.] = 597.000000
y[2][5][14][.] = 652.000000
y[2][5][15][.] = 708.000000
SSIJK = 53493392.000000
SSTO = 12391836.000000
SSA = 298996.000000
SSB = 1376796.000000
SSAB = 216188.000000
SSC(A) = 1825832.000000
SSBC(A) = 8674024.000000
MSA = 298996.000000
F(A) = 4.585246
— Writing combined dataset into ./groupftest+tlrc.HEAD
MSB = 344199.000000
F(B) = 4.444337
— Writing combined dataset into ./trialftest+tlrc.HEAD
MSAB = 54047.000000
F(AB) = 0.697861
— Writing combined dataset into ./gtftest+tlrc.HEAD

As indicated above, 139 MB of free disk space were required for program execution. Of this, 52 MB are required for the 3 permanent *AFNI* 'fift' datasets. (Of course, this is in addition to the 1300 MB required for the input datasets.)

From Table 4 we see that, for Model 5, the F-statistic to use for testing for the presence of factor A main effect is $F(A) = \frac{MSA}{MSC(A)}$. From the screen output (for voxel #1234567) we see that $SSA = 298996$ and $SSC(A) = 1825832$. Since SSA has $df = a - 1 = 1$ degrees of freedom, and $SSC(A)$ has $df = a(c-1) = 2(14) = 28$ degrees of freedom, the F-statistic is:

$$F(A) = \frac{SSA/(a-1)}{SSC(A)/(a(c-1))} = \frac{298996/1}{1825832/28} = 4.5852\ .$$

Similarly, one can verify that

$$F(B) = \frac{SSB/(b-1)}{SSBC(A)/(a(b-1)(c-1))} = \frac{1376796/4}{8674024/(2(4)(14))} = 4.4443,$$

and

$$F(AB) = \frac{SSAB/((a-1)(b-1))}{SSBC(A)/(a(b-1)(c-1))} = \frac{216188/4}{8674024/(2(4)(14))} = 0.69786\ .$$

Of course, these results apply only for the specified voxel. The $F(A)$, $F(B)$, and $F(AB)$ F-statistics for all $4,335,891$ voxels are stored in files groupftest+tlrc.BRIK, trialftest+tlrc.BRIK, and gtftest+tlrc.BRIK, respectively.

Note that since $n = 1$ (i.e., no replications) in this example, it is not possible to calculate $F(C)$ or $F(BC)$, as these statistics both require calculating SSE, which has 0 degrees of freedom. If the operator had used either the -fc command or the -fbc command in the batch command file, the program would have printed an error message and stopped.

From the screen output, one could construct the following ANOVA table:

ANOVA Table for Example 3, Voxel #1234567

Source of Variation	SS	df	MS	F*
Factor A	298996	1	298996	4.59
Factor B	1376796	4	344199	4.44
Factor C(A)	1825832	28	(65208)	—
A×B Interaction	216188	4	54047	0.70
B×C(A) Interaction	8674024	112	(77447)	—
Total	12391836	149		

As with the previous examples, the separate output datasets could be replaced with a single "bucket" dataset by adding the -bucket command to the batch command file.